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GENE INTERACTION IN TRYPTOPHAN SYNTHETASE FORMATION[1]

CHARLES YANOFSKY[2] AND DAVID M. BONNER

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Received March 21, 1955

IN a previous study (YANOFSKY 1952) of two biochemically similar tryptophan-requiring mutants of *Neurospora crassa* it was found that each strain differed from the wild type in that it was unable to carry out the tryptophan synthetase reaction (the coupling of indole and serine to form tryptophan). In both mutants the genetic change which resulted in a requirement for exogenous tryptophan seemed to be at the same locus (termed the *td* locus) and thus, on the basis of the initial biochemical and genetic criteria that were applied, the two strains appeared to be identical. The occurrence of a suppressor mutation (restoring the ability to grow in the absence of tryptophan) in one of the strains subsequently permitted the demonstration that the two mutants differed. Only one of the mutant strains responded to the presence of the suppressor gene by forming tryptophan synthetase and growing in the absence of a tryptophan supplement; the second strain, when it contained the suppressor gene, showed neither of these effects.

Since these observations indicate that suppressor gene specificity can be used to detect differences at the locus controlling the formation of tryptophan synthetase, additional mutants and suppressors have been obtained and examined. The behavior of these new mutants and suppressors is the subject of this paper.

TERMINOLOGY

Mutant-suppressor combinations that grow in the absence of an exogenous supply of tryptophan are called suppressed mutants; mutants which are capable of responding to specific suppressor genes are called suppressable mutants. Mutants which do not carry suppressor genes or are unaffected by the suppressor genes they carry are termed unsuppressed mutants; mutants that do not respond to any of the known suppressor genes are tentatively classified as unsuppressable mutants.

DESCRIPTION OF MUTANTS

Twenty-five tryptophan-requiring (indole non-utilizing) mutant strains, designated td_1 through td_{25}, (*td* strains are *tryp-3* strains in the terminology of BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954) were employed in this investigation. Of these, td_1 (C-83) was supplied by DR. H. K. MITCHELL of the California Institute of Technology and td_2 (S-1952) by DR. E. L. TATUM of Stanford University. Strains td_3 through td_{25} were obtained in this laboratory from ultraviolet irradiated macro-

[1] The work reported in this paper was supported in part by the Atomic Energy Commission, (Contract # AT(30-1)-1017), and in part by the American Cancer Society on recommendation by the Committee on Growth. A preliminary report of some of the material presented in this paper was published in the Records of the Genetics Society 22:112 (1953).

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conidia of a single stock, the ST. LAWRENCE wild type strain 74 A. Strain td_{24} was obtained by J. WEIJER. The filtration method of WOODWARD, DEZEEUW and SRB (1952) was employed in the isolation of the twenty-three mutants. Since these strains originated from a single stock, they are presumably isogenic except for the genetic change which resulted in a requirement for tryptophan. Mutant strains td_3 through td_{25} represent the yield from four separate searches.

The tryptophan requirement characteristic of each of the twenty-five *td* mutants cannot be satisfied by indole or anthranilic acid. All the strains respond approximately equally well to added tryptophan and, except for strains td_3 and td_{24}, they do not grow in the absence of an exogenous supply of this amino acid. Strain td_3 grows very slightly in the absence of tryptophan (in a two-week period) and strain td_{24}, a temperature sensitive mutant, will grow without added tryptophan at temperatures of 30°C or above. Extracts of the latter strain, as might be expected, exhibit tryptophan synthetase activity though the content of this enzyme is very low. It has not as yet been possible to detect tryptophan synthetase activity in extracts of any of the other twenty-four mutant strains. Details of the examination of extracts of strains td_1 and td_2 have been reported previously (YANOFSKY 1952).

Allelism tests of the various *td* strains suggest that all of these strains represent alterations of the same locus. Extensive evidence for the allelism of the mutant loci in strains td_1 and td_2 has been presented previously (NEWMEYER 1954; YANOFSKY 1952). Other members of the *td* series have also been examined, though in less detail (WEIJER 1954), and again the evidence suggests they are all allelic. In many of the interallele crosses that have been examined, tryptophan independent strains have been found among the progeny of the crosses. Such tryptophan independent strains occur infrequently. These *td* strains thus appear to resemble *Q* locus mutants (BONNER 1951; ST. LAWRENCE 1955). Crosses of $td_1 \times td_1$ have also yielded infrequent tryptophan independent progeny (ca. $1/10^4$ ascospores). The origin of such tryptophan independent progeny has not been established. In any event, however, it appears that the *td* strains represent either mutations of a common locus or mutations of closely linked interdependent units, with a common function.

ORIGIN OF THE SUPPRESSORS

Tryptophan independent (T^+) cultures could arise from tryptophan dependent (T^-) *td* mutants as a result of either reversion or suppression. To distinguish between these possibilities it is necessary to perform a cross. If, for example, a T^+ culture were crossed to wild type only $8T^+:0T^-$ asci would be formed if tryptophan independence resulted from reversion. If, however, suppression was responsible for tryptophan independence, $4T^+:4T^-$ and $6T^+:2T^-$ asci would be obtained in addition to the $8T^+:0T^-$ class. In such crosses the appearance of a $6T^+:2T^-$ ascus would be particularly significant for two reasons. First, since T^+ colonies derived from T^- macroconidia are frequently heterocaryons (with the T^- strain), $4T^+:4T^-$ asci would also be expected from a cross of wild type by a T^+ revertant colony. This leaves the 6:2 ascus as our only initial means of distinguishing between suppression and reversion. Secondly, a $6T^+:2T^-$ ascus is itself proof of suppression since in an ascus of this type one mutant spore pair must carry a suppressor while the second mutant spore pair does not.

It was reported previously (YANOFSKY 1952) that *td*$_2$ *su*$_2$ (each suppressor gene, i.e. *su*$_2$, is assigned the number of the *td* mutant in which it was discovered) exhibits a pronounced growth lag on a medium lacking tryptophan. This lag is especially noticeable on sorbose-minimal agar (TATUM *et al.* 1949). On this medium *td*$_2$ *su*$_2$ colonies derived from plated macroconidia become visible 3–4 days later than wild type colonies and are classified as delayed colonies. The *td*$_2$ *su*$_2$ colonies are morphologically distinguishable from the wild type colonies in that they consist of relatively few mycelial filaments and never give the thick, sharp-bordered appearance characteristic of wild type colonies. These differences between wild type and *td*$_2$ *su*$_2$ colonies, as it turned out, were helpful in the isolation of new suppressors.

A typical experiment designed to detect suppressor mutations was performed as follows: Portions of a suspension of macroconidia of a *td* mutant were plated on sorbose-minimal agar before and after ultraviolet irradiation (to obtain both spontaneous and induced suppressor mutations). Tryptophan independent colonies usually appeared on the plates after 2–3 days of incubation at 30°. These colonies were picked and transferred to minimal agar slants. The plates were reincubated for 4–5 days and if a second crop of colonies appeared they were also picked. This second crop usually contained a large proportion of delayed colonies. All the colonies which were picked were then crossed to wild type to distinguish between reversion at the *td* locus or suppressor mutations at other loci. From each cross asci were dissected in order and the cultures derived from the ascospores were tested to determine whether or not they required tryptophan.

The predominant ascus type encountered in most of the crosses contained four T^+ and four T^- spores. This was anticipated since the colonies obtained by the method employed were almost invariably heterocaryotic. Some crosses gave only $8T^+:0T^-$ asci in addition to the 4:4 type. This was taken as in indication that the tryptophan independence of the picked colony employed in the cross was the result of reversion at the *td* locus. An occasional cross gave $6T^+:2T^-$ asci in addition to the other two types. As mentioned previously, the 6:2 ascus establishes that an independently segregating gene, distinct from the *td* locus, is responsible for the tryptophan independence of two of the spores. Of the six T^+ cultures two usually showed a 3–4 day lag (in liquid minimal medium) and grew much slower than the other T^+ cultures. These slow growing T^+ cultures in 6:2 asci were presumably the suppressed mutants. To prove that they actually were suppressed mutants one spore of each spore pair was crossed to wild type (see crosses 1, 3, 5 and 6 in table 1). The recovery of mutant spore pairs from these crosses indicated that the slow growing parental strain was a suppressed mutant. In a number of cases the presence of a suppressor gene was confirmed by showing that the T^+ cultures derived from 4:4 asci were wild type strains carrying suppressors (see crosses 2, 4 and 7 in table 1).

It was mentioned previously that both fast growing colonies and delayed colonies were encountered in hunts for new suppressors. In our experiences the tryptophan independence of the fast growing colonies is inseparable from the *td* locus suggesting that in these colonies reversion is the cause of tryptophan independence. To date, well over 50 fast growing colonies derived from a number of different *td* mutants have been analyzed and, without exception, tryptophan independence has been inseparable from the *td* locus. The tryptophan independence of delayed colonies,

TABLE 1

Detection of suppressors in strains td_2, td_3, td_6 *and* td_{24}

	Cross	Ascus type		
		$8T^+$	$6T^+:2T^-$	$4T^+:4T^-$
1	$td_2\ su_2 \times$ wild type	4	17	5
2	$su_2 \times td_2$	13	42	19
3	$td_6\ su_6 \times$ wild type	2	9	3
4	$su_6 \times td_6$	5	11	3
5	$td_3\ su_3 \times$ wild type	1	12	0
6	$td_{24}\ su_{24} \times$ wild type	1	7	2
7	$su_{24} \times td_{24}$	2	8	0

T^+ = tryptophan independent T^- = tryptophan dependent

on the other hand, was frequently due to suppressor mutations. All suppressors obtained to date have been isolated from delayed colonies. Delayed colonies have not been obtained from all *td* mutants; some strains give only the fast growing type. In this connection it is interesting to note that td_1, a strain in which all attempts to isolate a suppressor have been unsuccessful, does not give delayed colonies.

SUPPRESSOR GENE SPECIFICITY

The specificity of the four suppressor genes was examined by crossing wild type strains carrying each of the suppressors to the four suppressable strains and to td_1, which was previously found to be unsuppressable by su_2 (YANOFSKY 1952). The results of these crosses are given in table 2. It can be seen that su_2 suppresses td_2 but does not suppress the mutant allele in any of the other strains. Suppressor$_6$ suppresses td_2 as well as td_6. Both td_3 and td_{24} are suppressed by su_3 and su_{24} but neither of these suppressors affects td_1, td_2 or td_6.

Several crosses were performed with two suppressors present to determine whether any of the suppressor genes are allelic and whether the presence of one suppressor gene would affect suppression by a second suppressor gene. The results of these crosses are given in table 3. In no case was there any indication that one suppressor gene inhibits the effect of a second suppressor gene. In the cross $su_6 \times td_2\ su_2$, as can be seen in table 3, the $8T^+:0T^-$ ascus class was very large. This was expected since both su_2 and su_6 suppress td_2. The occurrence of 6:2 and 4:4 asci in this cross indicates that su_2 and su_6 are not allelic. The absence of 6:2 and 4:4 asci from crosses involving both su_3 and su_{24} would suggest that these suppressors are allelic or very closely linked. The similarity of su_3 and su_{24} is also evident from growth studies performed with strains carrying these suppressor genes. These studies have shown that both $td_3\ su_3$ and $td_{24}\ su_{24}$, in contrast to other suppressed mutants, are temperature sensitive (in the absence of tryptophan they grow best at high temperatures, as does td_{1A}).

Two suppressor genes, su_2 and su_6, were crossed into all 25 of the *td* mutants to determine the extent of the specificity of these two suppressors. None of the *td* mutant alleles except td_2 (suppressed by su_2 and su_6) and td_6 (suppressed by su_6) was suppressed by either su_2 or su_6.

TABLE 2

Suppressor specificity crosses with strains td_1, td_2, td_3, td_6 *and* td_{24}

Cross	Ascus type		
	$8T^+$	$6T^+:2T^-$	$4T^+:4T^-$
$su_2 \times td_1^*$	0	0	59
$su_2 \times td_3$	0	0	5
$su_2 \times td_6^*$	0	0	19
$su_2 \times td_{24}$	0	0	12
$su_6 \times td_1^*$	0	0	26
$su_6 \times td_2$	1	5	0
$su_6 \times td_3^*$	0	0	5
$su_6 \times td_{24}$	0	0	11
$su_3 \times td_1$	0	0	10
$su_3 \times td_2$	0	0	12
$su_3 \times td_6$	0	0	5
$su_3 \times td_{24}$	1	2	2
$su_{24} \times td_1$	0	0	6
$su_{24} \times td_2$	0	0	10
$su_{24} \times td_3$	3	3	3
$su_{24} \times td_6$	0	0	17

T^+ = tryptophan independent T^- = tryptophan dependent

* The presence of the expected suppressor gene in the progeny of this cross was confirmed by an additional cross to a mutant affected by the suppressor.

TABLE 3

Crosses involving two suppressors

Cross	Ascus type		
	$8T^+$	$6T^+:2T^-$	$4T^+:4T^-$
$su_2 \times td_6\ su_6$	3	12	5
$su_6 \times td_2\ su_2$	10	9	3
$su_2 \times td_{24}\ su_{24}$	1	9	0
$su_6 \times td_{24}\ su_{24}$	0	16	2
$su_3 \times td_{24}\ su_{24}$	20	0	0
$su_{24} \times td_3\ su_3$	13	0	0

T^+ = tryptophan independent T^- = tryptophan dependent

Crosses were also carried out to examine the possibility that an unsuppressable mutant, such as td_1, does not respond to su_2 because it does not carry the proper modifier genes. A revertant derived from a suppressable td_2 stock and presumably carrying any and all modifiers necessary for suppression by su_2 was employed in these crosses. This revertant was crossed to td_1 and a td_1 stock obtained from the progeny. This td_1 was crossed to the revertant and a td_1 stock was again isolated from the progeny. The revertant was crossed to this new td_1 stock and a td_1 was selected from the progeny of this cross. This td_1 stock which was approaching isogenicity with the td_2 revertant, was then crossed to wt su_2. This new td_1, like the original parental td_1, was unaffected by su_2. Thus the inability of td_1 to respond to su_2 appears to be associated with the *td* locus.

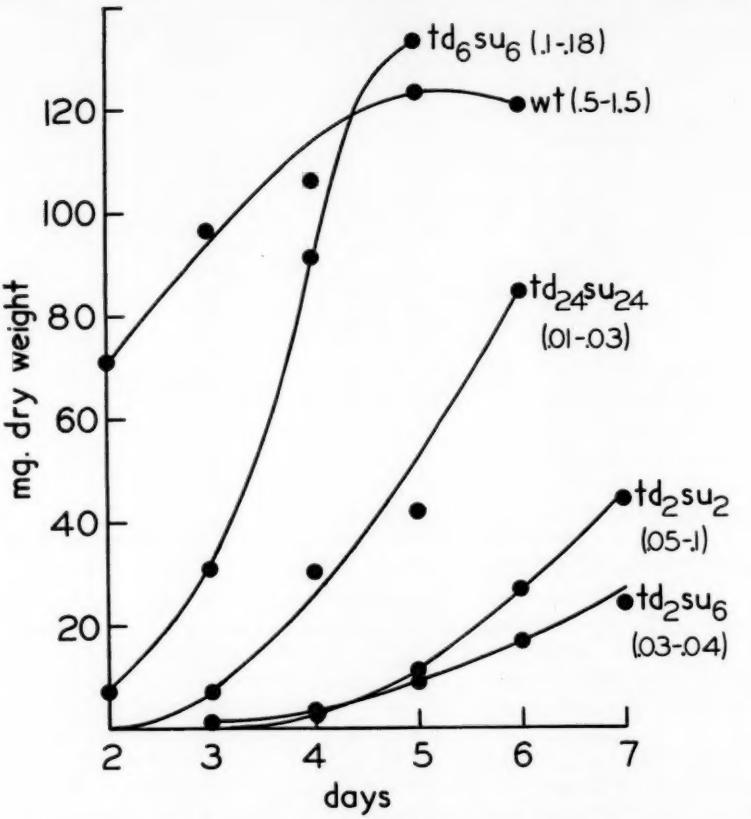

FIGURE 1.—Typical growth curves on minimal medium of several suppressed mutants and wild type. Specific tryptophan synthetase activities of the various strains are given in the parenthese after each curve.

CHARACTERISTICS OF SUPPRESSED MUTANTS

Although all suppressed mutants grow in the absence of tryptophan, each is stimulated markedly by this amino acid. In contrast to the mutants, however, low levels of tryptophan give maximal growth responses. A comparison of the growth curves on minimal medium of several of the suppressed mutants and of a typical wild type strain is shown in figure 1. From this figure it can be seen that there is a pronounced lag in the growth of suppressed mutants. The length of this lag is critically affected by inoculum size. In the presence of tryptophan, however, inoculum size is not a limiting factor.

Extracts of a number of the suppressed mutants have been examined for trypto-

TABLE 4

Comparison of the properties of tryptophan synthetase preparations from $td_2\ su_2$, $td_6\ su_6$ *and wild type*

	Wild type	*td_2 su_2*	*td_6 su_6*
pH optimum	7.8	7.8	7.6
Concentration required for half maximum velocity			
indole	5.6×10^{-5}M	4.8×10^{-5}M	5.3×10^{-5}M
L-serine	3.4×10^{-3}M	3.4×10^{-3}M	3.2×10^{-3}M
B_6alP	1.3×10^{-6}M	1.8×10^{-6}M	1.1×10^{-6}M
stability			
loss of activity at 2°C	over 50% in 24 hours	over 50% in 24 hours	over 50% in 24 hours
protected by	B_6alP+GSH	B_6alP+GSH	B_6alP+GSH
% inhibition by			
NH_2OH 10^{-3}M	92	100	100
CN^- 10^{-3}M	54	40	51
Co^{++} 10^{-3}M	76	73	87
Zn^{++} 10^{-3}M	81	78	61
L-tryptophan 2×10^{-3}M	29	36	29
Energy of activation	ca. 12,000 cal/mole	ca. 12,000 cal/mole	—
Fractionation behavior		same as wild type	same as wild type

B_6alP = pyridoxal phosphate
GSH = glutathione

phan synthetase activity and in every case the presence of the specific suppressor gene restored the ability to form this enzyme. However, in no case did the tryptophan synthetase content of a suppressed-mutant extract reach the wild type level. Typical tryptophan synthetase levels (specific activities) of several of the suppressed mutants are given in parentheses in figure 1. Partially purified tryptophan synthetase preparations from two of these suppressed mutants, *td_2 su_2* and *td_6 su_6*, have been compared with similar preparations from a wild type strain. A summary of the properties examined and the results obtained in these comparisons is given in table 4. It is apparent that no major differences were detected.

DISCUSSION

The data presented emphasize the biochemical similarities of the 25 *td* mutants. The genetic change in each of these mutants is expressed as a tryptophan requirement which cannot be satisfied by indole, and as an inability or limited ability to form tryptophan synthetase. Each of the *td* mutants presumably represents an independent occurrence of a mutation affecting tryptophan synthetase formation. The fact that so many independent occurrences of mutations affecting one enzyme appear to be at or near the same chromosomal area probably indicates that this area is most critically concerned with tryptophan synthetase formation. Mutations at other loci, if they also affect tryptophan synthetase formation, do not appear to be capable of reducing the level of this enzyme to a point at which it limits growth.

TABLE 5

Suppressor specificity

td	Suppressor			
	SM2	*SM3*	*SM6*	*SM24*
td_1	−	−	−	−
td_2	+	−	+	−
td_3	−	+	−	+
td_6	−	−	+	−
td_{24}	−	+	−	+

+ indicates suppression.

In spite of the apparent biochemical and genetic similarities of the *td* mutants, studies employing suppressor genes have permitted the detection of distinct differences between several of the *td* mutants. The suppressor genes were found to be highly specific (a summary of suppressor specificity is presented in table 5) and to suppress only certain members of the *td* series. Thus, although our present biochemical techniques do not enable us to demonstrate differences in the various *td* mutants, the suppressor studies show that these differences do exist. The conclusion is inescapable that the chromosomal area we call the *td* locus has been affected differently in different members of the *td* series.

In attempting to account for the results obtained two hypotheses are being considered. The first hypothesis assumes 1) the *td* locus, either directly or indirectly controls the synthesis of one enzyme, tryptophan synthetase, 2) many of the *td* mutants are blocked in different steps or different phases of tryptophan synthetase formation and 3) the suppressors provide specific substances which are capable of partially overcoming the defect in tryptophan synthetase formation in specific *td* mutants. The second hypothesis assumes that each *td* type produces a substance which specifically inhibits the formation of tryptophan synthetase. The suppressors would then act by blocking the synthesis of specific inhibitors. This hypothesis requires that the *td* locus control the formation of several different inhibitors, each specific in its action on tryptophan synthetase formation.

If the defects in the *td* mutants do affect tryptophan synthetase formation, as our results suggest, suppressable mutants might be expected to accumulate "unfinished enzyme". Some preliminary serological studies (SUSKIND, YANOFSKY and BONNER 1955) aimed at examining this possibility have shown that suppressable mutants do, in fact, form an antigen or antigens closely related to tryptophan synthetase while an unsuppressable strain, such as td_1, does not. Although the actual relationship of the antigen to tryptophan synthetase formation is at present unknown, the serological approach seems promising and may, in time, permit us to distinguish between the two hypotheses which are being considered.

SUMMARY

Twenty-five biochemically similar tryptophan requiring-strains have been studied in respect to their response to specific suppressor genes.

These mutant strains appear to be allelic and are altered in that each has a tryptophan requirement which cannot be satisfied by indole, and each is unable or has limited ability to form tryptophan synthetase.

Despite the apparent biochemical and genetic similarities of these strains, studies employing suppressor genes permit the detection of distinct differences between some of the strains. Thus the chromosomal area, termed the *td* locus, has been affected differently in different members of this allelic series.

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BLOOD GROUP FACTORS IN THE RABBIT. II. THE INHERITANCE OF SIX FACTORS[1]

CARL COHEN

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BLOOD groups in the rabbit have been studied genetically since CAMERON and SNYDER (1933), CASTLE and KEELER (1933) and KEELER and CASTLE (1933, 1934) used the two isoantibodies prepared by LEVINE and LANDSTEINER (1929, 1931) and FISCHER and KLINKHARDT (1929, 1930) to classify rabbits into four groups. By the use of these antibodies (called anti-H_1 and anti-H_2) animals were shown to be H_1, H_1H_2, H_2 or O. These studies clearly demonstrated that blood group factors H_1 and H_2 were controlled by a series of three alleles, giving rise to six different genotypes.

FISCHER later (1935) uncovered three more agglutinogens, K_3, K_4, and K_5. These differed in specificity from H_1 and H_2 and appeared to be controlled by genetic mechanisms, although no genetic tests were performed similar to those used by CAMERON *et al.* MARCUSSEN (1936) reported on the serologic behavior of rabbit isoantibody and postulated the discovery of further antigenic differences.

KNOPFMACHER (1942) did further genetic and serological work on the first two isoantibodies reported and investigated an isoantibody isolated by himself, the inheritance and specificity of which were independent of H_1 and H_2; he called this isoantibody anti-H_6.

More recently NACHTSHEIM and KLEIN (1948) reported a gene-controlled hydrops fetalis in the rabbit which was thought to be related to blood cell incompatibility. No specific blood group factor was implicated. HEARD, HINDE and MYNORS (1949), in an attempt to corroborate these findings, induced the production of isoantibody in the rabbit by repeated immunizations, but were unable to find any effects upon the offspring of incompatible matings. The mode of inheritance of the blood group factors detected by the antibodies isolated by these workers has not been reported. KELLNER and HEDAL (1953 a & b) have prepared two isoantibodies detecting two blood group factors, called *G* and *g*, which behave as if controlled by a pair of alleles and which are capable of causing incompatibility reactions.

The present study was undertaken in an effort to systematize the knowledge of blood groups in the rabbit through utilization of the large colony of rabbits which has been maintained at the Jackson Laboratory by DR. P. B. SAWIN. This report covers the second phase of the work (see COHEN 1955), namely, the study of the inheritance of the rabbit blood group factors.

Since none of the previously reported typing sera were available at the time this

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work was started, it was necessary to prepare our own typing sera and arbitrarily set up the system of nomenclature which will be more fully discussed later in this report.

In our analysis of the inheritance of rabbit blood groups we were interested in possible parallelism with the mode of inheritance of some of the human blood group systems: for example, the human ABO system, where there are three or more alleles at a single locus, or the MN type of inheritance of the MNS system, where both genes are detectable, or the P blood group, where P is inherited as a Mendelian dominant character and the recessive is not detectable. We were also interested in testing the possibility that a single allele gives rise to more than one blood group factor, as postulated for the mode of inheritance in the Rh system in humans (WIENER 1952) and in other species (STORMONT 1951; BRILES 1950).

MATERIALS AND METHODS

The immune testing sera were prepared by the immunization of rabbits with the whole blood of other rabbits. The methods and techniques are explained in detail in preceding publications (COHEN 1954, 1955). By testing the immune sera against a panel of a number of different rabbit bloods it was possible to establish the presence of antibody specific to some of the panel cells. Reacting panel cells were used for absorption, and the absorbed sera were again tested against the panel. From a total of 46 animals producing immune sera which were analyzed by absorption methods, 6 specific antibodies were prepared for differentiating the cells of the rabbit. The specific antibodies were given a letter symbol according to the order in which they were isolated in our laboratory. These symbols bear no relation to the symbols used to denote blood group systems in other animals or to typing sera previously prepared for the rabbit. Certain sera were adopted as the standards for each specificity. Thus serum from rabbit 1S8 which had been injected with the blood of rabbit 8M70 and then absorbed by the cells of rabbit 1P14 is designated A. The following designations are based upon this scheme:

Donor	*Recipient*	*Absorbing cell*	*Symbol*
8M70	1S8	1P14	A
2095	7N50	1N40	B
2095	7N50	9R84	C
3M67	5M57	3R03	E
1N62	3S29	2095	F
3S46	3Q91	7N43	H

The six specific antibodies appear to be monospecific because of the distribution of positive reactions in over 500 animals tested. Table 1 shows the result of a test of a selected group of animals and indicates the specificity of each serum.

The genetic tests made were based upon matings of animals whose blood types proved interesting. In most cases the matings were set up so that the equivalent of backcrossing was carried out; thus, animals with certain blood group factors were mated to animals lacking the factor or factors of interest. The blood types of the offspring were determined, and the kinds and number of classes of offspring were used as indicators of the mode of inheritance.

TABLE 1

Blood types of selected rabbits as indicated by agglutination of their red blood cells by specific typing sera

Rabbit no.	Blood type	Typing serum					
		A	B	C	E	F	H
7023	A	+	−	−	−	−	−
3W51	B	−	+	−	−	−	−
9U60	C	−	−	+	−	−	−
4P65	E	−	−	−	+	−	−
1N62	F	−	−	−	−	+	−
9U63	H	−	−	−	−	−	+
9R84	AB	+	+	−	−	−	−
6Y78	BC	−	+	+	−	−	−
1T60	ABCEH	+	+	+	+	−	+
223	ACEFH	+	−	+	+	+	+
95	BE	−	+	−	+	−	−
2T13	ABCEF	+	+	+	+	+	−
2T15	ABCEFH	+	+	+	+	+	+
3S29	O	−	−	−	−	−	−

RESULTS

Because animals of our colony are descended from the animals used by CASTLE and KEELER in their early studies on the inheritance of blood groups in the rabbit, it was expected that some of the isoantibodies prepared by us would be similar to anti-H_1 and anti-H_2. Since the most commonly produced antibodies resulting from all our immunizations were anti-A and anti-F, it was felt that these antibodies might be H_1 and H_2. On the basis of the knowledge that H_1 and H_2 were part of a three allele system (wherein the O type occurs rarely), it was our first object to test the allelism of the genes responsible for A and F. Since our population is not truly random and the bulk of the more than 500 animals tested belonged to three relatively closely bred lines, we could not apply random population statistics, and therefore had to rely wholly upon breeding data. Table 2 shows the results of breeding animals with particular reference to blood types A and F and the lack of both. It can be seen that if these two factors were controlled by alternative alleles there could be only two classes of offspring resulting from a cross involving an animal having factors A and F and an animal which has neither factor. Line 7 of table 2 shows matings of this sort. Examination of lines 4, 5, 6, and 7 discloses that in any cross involving an animal which has A and F no offspring lacks both A and F. A third allele is indicated in matings shown in lines 2, 3, 7, and 9; namely, an allele which is neither A nor F and is apparently recessive to both.

Table 3, showing the results of four random matings, indicates again by the number and classes of offspring that factors A and B are not controlled by alleles, since a mating of an animal of type AB with an animal which has neither A nor B gives rise to animals which have neither factor A nor B.

With the results indicated in tables 2 and 3 in mind, examination of table 4, which

TABLE 2

Test of allelic control of factors A and F. Hypothesis—A and F are controlled by allelic genes

	Parent types	Rabbit no.	Offspring types					
			No.	A	AF	F	Neither	
1	A × A	1V0 × 1V3	19	14	—	—	5	
2	A × O	7K86 × 3L96	12	12	—	—	—	
		1T57 × 2J03	14	6	—	—	8	
3	O × O	9U63 × 9U60	5	—	—	—	5	
		4 × 9U60	6	—	—	—	6	
4	AF × AF	4R27 × 4R21	4	1	1	2	—	
		4R24 × 4R21	6	3	2	1	—	$\chi^2 = 1.91$
		220 × 223	8	1	4	3	—	df = 2
		221 × 223	6	0	3	3	—	
5	a AF × A	2V85 × 2U87	5	2	3	—	—	
	b	A67 × A68	8	6	2	—	—	
	c	2T15 × 1S81	14	8	1	5	—	$\chi^2 = 2.86$
								df = 1
6	AF × F	6R06 × 6U05	2	—	2	—	—	
7	AF × O	2T13 × 2J03	4	1	—	3	—	
		84 × 4R44	9	4	—	5	—	$\chi^2 = 2.25$
		173 × 4R44	3	—	—	3	—	df = 1
8	F × F	5R75 × 5R74	8	—	—	5	3	
		6061 × 5036	7	—	—	5	2	
9	F × O	6J33 × C10-11	5	—	—	5	—	
		TCl-14 × TCl-12	8	—	—	5	3	
10	F × A	5R75 × 1S81	4	1	1	1	1	

TABLE 3

Test of allelic control of factors A and B

	Parents			Offspring				
	Type	Number		No.	Type			
		♀	♂		A	AB	B	Neither
1	AB × A	2715	1S81	14	5	3	4	2
2	AB × —	1T57	2J03	14	3	3	3	5
3	AB × —	2T13	2J03	4	—	1	2	1
4	A × B	5043	5040	3	1	2	—	—

TABLE 4

Test of allelic control of 6 blood group factors by examination of 14 offspring resulting from the mating of 2T15 (♀ type ABCEFH) and 1S81 (♂ type AC)

A. Kinds of offspring produced

ABCE
ABCEF
ABE
ABEH
ACEH
AE (2)
AEH (2)
BCEF
BCEFH
BEF
CEF
EFH

B. Frequency of each factor

A	9
B	7
C	6
E	14
F	6
H	6

C.

	Test of allelism between gene controlling		No. of offspring in each class			
	X	Y	X only (A)	Y only (B)	Both XY (C)	Neither (D)
1	A	B	5	3	4	2
2	A	C	6	3	3	2
3	A	E	—	5	9	—
4	A	F	8	5	1	—
5	A	H	5	2	4	3
6	B	C	3	7	4	5
7	B	E	—	2	7	—
8	B	F	3	2	4	5
9	B	H	5	4	2	3
10	C	E	—	8	6	—
11	C	F	2	2	4	6
12	C	H	4	4	2	4
13	E	F	8	—	6	—
14	E	H	8	—	6	—
15	F	H	4	4	2	4

is the summation of the results of a mating of two selected animals, shows that all blood group factors other than A and F so far uncovered in our rabbits are inherited as independent characters. By the process of elimination of all possible allele pairs, it is clear that factors B, C, E, and H show the recombination characteristic of independent loci.

The number and classes of offspring of other matings, the results of which are used in the analysis for other mechanisms of inheritance, are shown in tables 5 and 6.

DISCUSSION

The data collected were used to test the following three hypotheses for the mode of inheritance of the blood group factors in the rabbit: (1) The presence or absence of each blood group factor is controlled by a single locus which has either of two alleles;

TABLE 5

Offspring resulting from a mating of IV0 (♀) type ABCE and IV3 (♂) type ACE

A. Kinds of offspring produced (20)

ABC	(1)	ACE	(2)
ABCE	(5)	ABE	(1)
AC	(5)	BC	(1)
AE	(1)	BCE	(2)
		CE	(2)

B.

Test of allelism between gene controlling		No. of offspring in each class			
X	Y	X only	Y only	XY	Neither
A	B	8	3	7	2
A	C	2	5	13	0
A	E	6	4	9	1
B	C	1	9	9	1
B	E	2	5	8	5
C	E	7	2	11	0

TABLE 6

Results of various matings showing classes of offspring produced

♀ No.	Blood type	♂ No.	Blood type	Factors of interest		No. of offspring	Classes of offspring produced			
				X	Y		X	Y	XY	neither
6J33	CE	C10-11	F	C	E	5	1	1	2	1
5R75	CEFH	1S81	AC	E	F	5	2	1	1	1
7X77	ABCEH	5X52	AC	E	H	6	3	2	0	1

the product of one allele is detectable and that of the other is not, as in the inheritance of the human P factor. (2) The factors are controlled by alleles such that two or more detectable factors are controlled by a single locus, as in the ABO system. (3) The factors are controlled by genes which give rise to more than one blood group factor, as postulated by WIENER for the Rh system.

1. Test of allelic control of any pair of blood group factors

From the results shown in table 2, we may state with some degree of safety that factors A and F are controlled by allelic genes and that a third allele, not as yet detectable by serologic methods, also exists at that locus. The data in table 2 were used for statistical tests of the hypothesis of allelic control in animals of group 4, group 5C, and group 7. Using χ^2 we cannot reject the hypothesis of allelism. However, by combining groups 4 and 7 we can test the hypothesis that A and F are at independent loci. We can reject this hypothesis with a high degree of confidence ($P < .01$).

Table 3 illustrates, with factors A and B, the methods used to test for allelism of the genes controlling all the blood group factors. The analysis is based upon the number and kinds of classes resulting from crosses of animals, with reference to the

occurrence of the factors of interest in the offspring. Efforts were made to determine whether in crosses involving animals of type AB and animals having neither A nor B there will occur offspring which have neither A nor B. If there are animals which do not have either, we can state that A and B are not alleles. Similar tests were made to test the relationship between each possible allelic pair, using a large number of litters for our conclusions. We were fortunate, however, in that one selected mating provided the information necessary to disprove the hypothesis of allelic control for any pair of factors other than AF. The results of this one mating, which yielded 14 offspring in two litters, are useful in showing in summary form the analysis made for tests of allelism of blood group factors in the rabbit, as well as for the test of whether one gene may give rise to more than one factor. The analysis made of table 4 is based upon the number and kinds of classes resulting from the cross of an animal of type ABCEFH and an animal of type AC. If four classes of offspring are obtained with reference to two of the tested blood group factors, we may assume that the two factors being examined are not controlled by alleles. The four classes can result, in this mating, only from recombination involving two loci. The analysis of table 4 is as follows:

If factors A and B are controlled by allelic genes, no animal can be produced which has neither A nor B. Since one parent (2T15) is type AB and the genotype with reference to A and B would be X^{A}/X^{B} (We will use the blood group factor symbol as a superscript to represent the gene at this time.), no offspring should lack both factors. Two such animals were found (line 1, column D). Similarly, tests of allelic control of AC, AH, BC, BG, BH, CF, and FH show that since animals do occur which have neither of the two factors of interest, we can assume that none of the above pairs are controlled by allelic genes. With reference to factor E, we may assume that 2T15 is homozygous for genes controlling E, since all offspring have factor E. The probability of all the offspring having E when 2T15 is heterozygous is less than .001. The homozygosity for E would preclude allelism of the gene controlling E and the gene controlling any other blood group factor, since an animal could not be homozygous and still show segregation at that locus. (Furthermore, tables 5 and 6 indicate that A and E, B and E, C and E, and H and E are not alleles.)

2. Test of plurality of factors controlled by a single gene

The test of the last hypothesis, namely, that a single gene may give rise to more than one factor, can be made in part by using the types of offspring resulting from the mating of 2T15 with 1S81. The interpretation in tabular form (table 7) uses the symbol for the blood group factor as superscripts on another letter to represent the gene. When the hypothesis indicates that more than one factor is controlled by the gene, the two letters representing the phenotype are shown as superscripts. Thus gene X^{AB} controls the presence of blood group factors A and B; gene X^{A} and gene X^{B} each give rise to one factor; and x denotes the lack of each of the two factors of interest.

To complete the interpretation the following assumption is necessary: if any of the offspring lack both of the factors of interest it must be the result of both parents having the recessive allele; that is, the allele which does not give rise to a detectable

TABLE 7

Interpretation of results shown in tables 4, 5, and 6 in terms of the one gene more than one antigen hypothesis

Test pair	Possible genes	No. animals with neither factor	Possible genotypes		Phenotypic classes		Reference table
			♂	♀	Theoret.	Actual	
AB	X^A, X^B, X^{AB}, x	2	X^{AB}/x	X^A/x	AB, A, O	AB, A, B, O	4
AC	X^A, X^C, X^{AC}, x	2	X^{AC}/x	X^{AC}/x	AC, O	A, C, AC, O	4
AE	X^A, X^E, X^{AE}, x	1	X^{AE}/x	X^{AE}/x	AE, O	A, E, AE, O	5 (IV0 × IV3)
AF	X^A, X^F, X^{AF}, x	0	X^{AF}/x or X^{AF}/X^A or X^{AF}/X^F	x/x	AF, O, AF, A, AF or F	A, F	2 (group 7)
AH	X^A, X^H, X^{AH}, x	3	X^{AH}/x	X^A/x	A, AH, O	A, H, AH, O	4
BC	X^B, X^C, X^{BC}, x	5	X^{BC}/x	X^C/x	BC, C, O	B, C, BC, O	4
BE	X^B, X^E, X^{BE}, x	5	X^{BE}/x	X^E/x	E, BE, O	B, E, BE, O	5 (IV0 × IV3)
BF	X^B, X^F, X^{BF}, x	5	X^{BF}/x	x/x	BF, O	B, F, BF, O	4
BH	X^B, X^H, X^{BH}, x	3	X^{BH}/x	x/x	BH, O	B, H, BH, O	4
CE	X^C, X^E, X^{CE}, x	1	X^{CE}/x	x/x	CE	C, E, CE, O	6 (6J33 × C10-11)
CF	X^C, X^F, X^{CF}, x	4	X^{CF}/x	X^c/x	C, CF, O	C, F, CF, O	4
CH	X^C, X^H, X^{CH}, x	4	X^{CH}/x	X^c/x	C, CH, O	C, H, CH, O	4
EF	X^E, X^F, X^{EF}, x	1	X^{EF}/x	x/x	EF, O	E, F, EF, O	6 (5R75 × 1S81)
EH	X^E, X^H, X^{EH}, x	1	X^{EH}/x	x/x	EH, O	E, H, O	6 (7X77 × 5X52)
FH	X^F, X^H, X^{FH}, x	4	X^{FH}/x	x/x	FH, O	F, H, FH, O	4

factor. Thus, since 2T15 is type ABCEFH and 1S81 is type AC, and since there are offspring which lack both A and B, then the genotype of 2T15 with reference to AB must be X^{AB}/x. Similarly, the genotype of 1S81 would be X^A/x, since factor B was absent.

The hypothesis that one gene gives rise to more than one factor, as applied to rabbit blood group factors, cannot be tested completely with this one family, since E appears to be homozygous in one parent and may be associated with another of the antigens. We must, then, turn to four other matings to test the relationship of E to each of the other blood group factors. Tables 5 and 6 show some other matings which serve as examples and which allow E as well as other types to be analyzed in a manner similar to that used for the family shown in table 4. The analysis of these matings is included in table 7. The results analyzed in table 7 clearly show that there is no case where a single gene appears to give rise to more than one blood group factor. If A and F were controlled by a single gene, then as a result of the matings shown in table 2, group 7, it would be expected that approximately one half of the total offspring would be AF. Since none out of 16 were AF, A and F in these cases could not be controlled by a single gene ($P < .01$).

The conclusion to be drawn from the above analysis is that there are five loci controlling the six blood group factors in the rabbit as studied by us. Factors A and F are controlled by allelic genes, with a third allele, determining an as yet undetectable factor, also present at that locus. All other factors—B, C, E, and H—are independent of A and F and of each other, and appear to behave as if controlled by simple dominant genes.

Through the courtesy of DR. AARON KELLNER we obtained samples of the two sera which he has been studying with reference to hemolytic diseases in the rabbit.

Comparison of his sera with ours shows that the sera designated by us as anti-F is the same as KELLNER's anti-G; similarly, our anti-A is the same as his anti-g. The results obtained using our sera, together with KELLNER's results, show that there are two demonstrable alleles at this one locus. We can go one step further and say that a third allele exists which is recessive to the other two and which does not give rise to a demonstrable antigen, since more than 70 of 500 rabbits tested do not have either A or F. It is possible that the large and diversified groups of rabbits available to us increased the probability of finding the third allele.

There is at the present time no uniform system of nomenclature for blood groups in the rabbit. A system which is compatible with existing genetic usage and blood group terminology is obviously necessary. Since none of the previously reported sera, other than KELLNER's, were available for comparison with our newly prepared sera, we are forced to discard the old nomenclature involving the use of *H* and *K* and develop a new system in which the genetic information is consistent with our immunologic knowledge.

Because most of the letters of the alphabet have been used to designate the various genes heretofore identified in the rabbit, we cannot use the blood group factor symbols to designate the genes controlling the blood group factors. We must, therefore, use a system involving at least two letters. Previous workers have used the letters *H*, *K*, and *G* to designate the genes involved, but certain inconsistencies from the point of view of genetic nomenclature have become apparent. A system is therefore proposed which encompasses earlier findings in rabbit blood group genetics, is compatible with the system of nomenclature applying to mammalian genetics, and gives direct indication of both phenotype and genotype in the symbolism.

The letter *H* is used to denote a detectable blood group locus. The small second letter describes the particular locus. A small *h* in the first position indicates an allele determining no detectable factor (a recessive notation). A superscript consisting of a capital letter denotes the particular allele in instances where more than two alleles are shown to exist at one locus.

Gene	*Blood group factor*
Hg^A	A
Hg^F	F
hg	neither
Hb	B
hb	lack of B
Hc	C
hc	lack of C
He	E
he	lack of E
Hh	H
hh	lack of H

The letter *H* was chosen because it was the first symbol used in the classical work of CASTLE and KEELER. Since reference to *G* and *g* has been made by KELLNER, we incorporate this notation in the three-allele series. It is suggested that as new iso-

antibodies are prepared the corresponding factor be given the next letter symbol in the alphabet. The gene (if it is independent of the known loci) then becomes *H* plus the small letter corresponding to the symbol of the new factor. If subsequently prepared antibodies disclose a relationship between two factors such that they are controlled by a single gene, then the superscript will show the two factors; e.g., Hx^{XY}.

As an example of the operation of this proposed system of nomenclature, the genotypes of the two parent animals shown in table 4 are as follows: 2T15—Hg^A Hg^F, *Hb hb*, *Hc hc*, *He He*, *Hh hh*; 1S81—Hg^A *hg*, *hb hb*, *Hc hc*, *he he*, *hh hh*.

Because of the large size and the genetic stability of the animal population in our laboratory, we have been able to set aside animals as standards which have only factor A, only factor C, only factor E, only factor F, and only factor H. Although at present these families are small, in time we expect to have each blood group factor segregated in an isolated line. It is proposed that these stocks be used as a standard of reference for making comparisons with blood group factors found in other laboratories.

SUMMARY

The inheritance of 6 blood group factors in the rabbit was studied. Two of the factors, A and F, are controlled by a single locus; a third allele at this locus produces no demonstrable antigen. All other factors are inherited independently as simple dominants, with the recessive giving rise to an as yet undetectable product. A system of nomenclature is proposed which is compatible with existing genetic usage and blood group terminology.

ADDENDUM

Since the acceptance of this paper for publication, an antibody has been prepared which is capable of detecting the antigen controlled by the gene *hg*. The antibody is designated as anti-D, and the corresponding antigen, D. This is the same antigen-antibody combination recently reported by JOYSEY 1955 (J. Exp. Biol. **32**: 440–449) as anti-G^e and G^e. Breeding experiments support the contention that all animals must have antigen A, D, or F, or any pair of these three. The *Hg* locus is now represented by the alleles Hg^A, Hg^D, and Hg^F, which give rise to antigens A, D, and F, respectively. The allele designated *hg* in the body of this paper has no place in the notation at this time.

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GENETICS OF NATURAL POPULATIONS. XXIII. BIOLOGICAL ROLE OF DELETERIOUS RECESSIVES IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA[1]

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GENES, or polygene complexes, which are deleterious in double dose occur in heterozygous condition in natural populations of every species of Drosophila so far studied in this respect. There is good reason to believe that harmful recessives are frequent in heterozygous condition in many, and possibly in all, Mendelian populations in which inbreeding is not too prevalent. The biological role played by the deleterious recessives concealed in natural populations is, however, not understood. DOBZHANSKY and WRIGHT (1941) and WRIGHT, DOBZHANSKY, and HOVANITZ (1942) inferred that at least some of the apparently recessive lethals in populations *of Drosophila pseudoobscura* are to some extent deleterious also in heterozygotes. Certain alternative interpretations, which would have made the assumption of an incomplete recessivity of the lethals unnecessary, were, however, not excluded. STERN *et al.* (1952) showed that most of the sex-linked lethals arising in laboratory cultures of *D. melanogaster* were incompletely recessive, and WALLACE and KING (1952) found a like situation with autosomal lethals. CORDEIRO (1952) and PROUT (1952) adduced similar evidence for naturally occurring autosomal lethals in *D. willistoni*. MULLER (1950) advanced theoretical arguments to show that recessive hereditary defects in man should produce mildly incapacitating effects in heterozygotes.

However, not all lethals affect adversely the fitness of heterozygotes. Heterotic lethals were found in *D. melanogaster* by MASING (1938), TEISSIER (1942), WALLACE and KING (1952), and STERN *et al.* (1952). CORDEIRO and DOBZHANSKY (1954) observed that at least 4 out of 52 second-chromosome lethals found in natural populations of *D. willistoni* were heterotic in combination with some other chromosomes from the same populations. GUSTAFSSON (1946) and GUSTAFSSON, NYBOM, and WETTSTEIN (1950) discovered mutants in barley which were poorly viable in homozygotes but advantageous in heterozygotes. For other examples, see LERNER (1954). More data on the effects in heterozygous condition of various classes of genetic variants which occur in natural populations are obviously needed.

The most important gap in present knowledge is the complete dearth of information on the fitness of heterozygotes for genes and gene combinations which are not lethal but only mildly deleterious when homozygous. It is now becoming customary to distinguish complete lethals (genotypes which make the viability of their carriers equal to zero in a certain environment), semilethals (viability above zero but less than 0.5 of the average for a given population), subvitals (viability above 0.5 but sig-

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nificantly less than 1.0), normals (viability within two standard errors from 1.0), and supervitals (viability significantly above 1.0). For more precise definitions of subvitality and supervitality, and for a description of methods used to estimate the frequencies of subvital and supervital chromosomes, see WALLACE and MADDEN (1953) and DOBZHANSKY and SPASSKY (1953). The present article reports the results of experiments comparing the behavior of subvital and supervital chromosomes found in a natural population of *D. pseudoobscura*. We hope to show that there is no correlation between the viabilities of homozygotes and heterozygotes for such chromosomes, and that combining different chromosomes from the same population results in a great variety of reaction norms the adaptedness of which varies in different environments.

VIABILITY OF HOMOZYGOTES IN A TEXAS POPULATION

Samples of the population of *D. pseudoobscura* were collected in the neighborhood of Austin, Texas, in March–May of 1953 by one of the authors (TH.D.) in cooperation with DRS. M. J. D. WHITE, M. R. WHEELER, MESSRS. W. HEED, L. METTLER, and other colleagues. The wild males, or single sons of wild females, were crossed (in the laboratory in New York) to laboratory strains with suitable mutant markers used to analyze the viability of the homo- and heterozygotes for second chromosomes in *D. pseudoobscura*. This experimental procedure has been described by DOBZHANSKY and SPASSKY (1953) and in earlier papers, and need not be re-described here in detail. Its essence is that individuals homozygous for a given wild chromosome are obtained in cultures which produce also flies heterozygous for the same chromosome and for another chromosome carrying a dominant mutant gene marker (Bare = short bristles). This is done by intercrossing females and males heterozygous for a certain wild chromosome and a chromosome with Bare and a crossover suppressor. Provided that the homozygotes and the Bare heterozygotes are equally viable, the offspring of the intercross should consist of 33.3% non-Bare flies (homozygotes) and 66.7% Bare heterozygotes. In reality, the proportion of non-Bare flies varies in different cultures from 0 (if a wild chromosome is lethal to homozygotes) to 33.3% and above. The data actually obtained in the experiments with the chromosomes from Texas are summarized in table 2. These data were obtained in cultures raised in incubators at 25°C, and on culture medium inoculated with Fleischmann's yeast.

A control experiment consists in intercrossing Bare females and Bare males heterozygous for second chromosomes derived from *different* wild flies. The non-Bare flies obtained in the offspring are heterozygotes which carry two second chromosomes of different derivation. The viability of such flies is normal by definition (see DOBZHANSKY and SPASSKY 1953). The control data are reported in table 1. It can be seen that the mean frequency of non-Bare flies in control progenies is $34.20 \pm 0.26\%$. This is slightly but significantly higher than the ideal 33.33%. It follows that the Bare chromosome depresses slightly the viability of its carriers.

The data in table 2 may now be considered. The 27 wild second chromosomes which have produced test cultures with no non-Bare flies are evidently lethal when homozygous. Test cultures with more than 0 but less than 17.1% of non-Bare flies (half of the control value, 34.2%) contain semilethal second chromosomes; such chromosomes numbered 29. The lethal and semilethal chromosomes together were

TABLE 1

Percentages of non-Bare flies in 110 intercrosses which produce heterozygous flies

Percent non-Bare	Intercrosses	Percent non-Bare	Intercrosses
29	5	37	16
31	15	39	4
33	37	41	2
35	29	43	2

Mean percentage 34.20 ± 0.26%.

TABLE 2

Viability of homozygotes for 178 second chromosomes derived from the Texas population. The figures show the numbers of chromosomes that produce cultures with different percentages of non-Bare flies

Percent non-Bare	Chromosomes	Percent non-Bare	Chromosomes
0	27	19	2
1	7	21	2
3	2	23	3
5	1	25	7
7	6	27	10
9	6	29	21
11	0	31	31
13	2	33	26
15	2	35	12
17	3	37	7
		39	1
Total lethals & semilethals	56	Total quasi-normals	122

56, or 31.5 ± 3.5% of the total. The remaining 122 chromosomes gave test cultures with from 19% to 39% non-Bare flies; these chromosomes are designated "quasi-normals." Among the quasi-normals, there are included subvitals, normals, and supervitals.

THE SUBVITAL AND SUPERVITAL CHROMOSOMES

Among the 178 chromosomes the performance of which in the homozygosity tests is reported in table 2, we have chosen, for the experiments to be described below, 10 chromosomes which gave cultures with between 18.5% and 25% of non-Bare flies, and 10 chromosomes which gave cultures with more than 35% of non-Bare flies. The former are presumably subvital and some of the latter supervital and others normally viable when homozygous. The percentages are based on counts of several hundred flies, and thus have a standard error of about 2%. To reduce this error further, we re-tested these chromosomes by raising more cultures, all of them at 25° and on Fleischmann's yeast. One of the subvitals proved to contain a peculiar condition expressed in production of varying proportions of dwarf flies. This condition was caused possibly by transmission of an infecting agent. This chromosome was eventually lost. The summary records of the other chromosomes are shown in table 3.

TABLE 3

Viability records of the subvital and supervital chromosomes used in the experiments

Supervitals			Subvitals		
No. of the chromosome	Flies counted	Percent non-Bare	No. of the chromosome	Flies counted	Percent non-Bare
10	2743	36.1	81	1264	28.6
158	4843	34.9	14	1438	28.4
62	3495	34.8	120	1502	27.6
202	3123	34.1	121	1400	27.6
150	5024	33.8	39	2037	27.1
57	1914	33.6	53	1530	25.8
1	3519	33.4	43	1948	23.7
106	1688	32.8	31	1878	22.4
16	1971	32.2	140	1471	16.2
50	2026	31.5			

The homozygotes for the chromosomes Nos. 10, 158, 62, and 202 may be classed as normal or supervital under the conditions of the experiment, and Nos. 150, 57, 1, 106, 16, and 50 as normally or very slightly subnormally viable. These ten supervital and normal chromosomes produce, then, homozygotes of high viability, and will be symbolized below by an H. Chromosomes Nos. 81, 14, 120, 121, 39, and 53 are mildly subvital, Nos. 43 and 31 strongly subvital, and No. 140 falls just within the conventional range of semilethality. These nine chromosomes will be referred to as "low" and symbolized by an L.

The 19 chromosomes listed in table 3 are perpetuated in the laboratory as balanced strains; the balancing chromosome has the dominant marker Bare, the recessive glass, and an inversion which suppresses most of the crossing over (see DOBZHANSKY and SPASSKY 1953).

The main experiment

Crosses were arranged to produce homozygotes for high (ten crosses), homozygotes for low (nine crosses), and heterozygotes for the following 27 combinations of H and L chromosomes:

H × *H*	*H* × *L*	*L* × *L*
1 × 10	1 × 14	14 × 31
10 × 16	10 × 31	31 × 39
16 × 50	16 × 39	39 × 43
50 × 57	50 × 43	43 × 53
57 × 62	57 × 53	81 × 120
62 × 106	106 × 81	120 × 121
106 × 150	150 × 120	121 × 140
150 × 158	158 × 121	140 × 14
158 × 202	202 × 140	
202 × 1		

All these 46 crosses were arranged in a similar way. Namely, groups of 12 females and 12 males carrying the Bare-glass-Inversion chromosome and the desired wild

second chromosomes were placed together in bottles with the standard cream of wheat-molasses medium used in the laboratory. The parents were transferred every day to fresh culture bottles, so that the oviposition period in each culture was limited to about 24 hours. Thus many bottles with eggs representing the progenies of the same group of parents were obtained. Each bottle was then subjected to one of the following four conditions: (a) incubator at 25°C; (b) constant temperature room at 16°; (c) 3 days at 16°, followed by 4 days at 4°, by 1 day at 25°, by 3 days at 16°, by 4 days at 4°, etc., until all the flies emerged; this variable temperature series is recorded in the tables below as "4°"; (d) incubator at 27° until the adults began to emerge, then at 25°. Equal numbers of cultures were placed in each of these four environments and the bottles obtained on successive days were always placed in different environments.

The first series of experiments under these four conditions was made with non-autoclaved food inoculated with Fleischmann's yeast (F). The second series used the yeast *Kloeckeraspora apiculata* (K), and the third series the yeast *Zygosaccharomyces dobzhanskii* (Z). These yeasts were supplied by PROFS. H. PHAFF and A. B. DA CUNHA, and were used in some previous work (e. g., DOBZHANSKY and SPASSKY 1954). For the experiments with these yeasts, the bottles with the Drosophila culture medium were autoclaved, on cooling inoculated with a suspension of the proper yeast, and left stoppered for 3 days to let the yeast grow before the flies were introduced.

In all series of experiments, 6 replicate bottles were used for each temperature-yeast combination. When the flies began to emerge, counts were made at 3-day intervals until the eclosion was complete or until there was a possibility that the next generation was appearing. All cultures were made moderately overpopulated; the mean number of hatching adults counted per culture (for 2214 cultures) was 135.5, the numbers being in general highest at 16° and lowest at 27°. The total number of flies counted in this main experiment was 299,508. The results are summarized in table 4.

In table 4 are given the numbers of flies counted and percentages of the non-Bare class in the totals for each series of experiments. Now, as indicated, above, we have studied the homozygotes for 10 different supervital (H) and 9 different subvital (L) chromosomes, and also the heterozygotes for, respectively, 10, 9, and 8 combinations of H × H, H × L, and L × L chromosomes. Since the different chromosomes and chromosome combinations yield different viabilities, table 4 shows also the chi-squares, degrees of freedom (df) and probabilities (P) of obtaining heterogeneities as large as those observed from sampling errors.

Influence of temperature on homozygotes

As stated above, the ten "high" and nine "low" chromosomes were selected because they produced, in cultures fed on Fleischmann's yeast and at 25°C (F 25° in table 4 and in figures 1 and 2), highly viable and subvital homozygotes respectively. The viability of the homozygotes for each of these chromosomes under these conditions is shown in figures 1 and 2 by black squares (cf. also table 3). It is obviously desirable to know how viable are these homozygotes under environmental conditions other than those in which they were selected. Table 4 and figures 1 and 2 supply the answer.

TABLE 4

Numbers of the flies counted and percentages of non-Bare flies among them. Experiments at different temperatures, and with food inoculated with Fleischmann's yeast (F), with Kloeckera spora (K), or with Zygosaccharomyces (Z) yeasts. In the H and L series the non-Bare flies were homozygous for chromosomes giving respectively high or low viability in double dose; in the H × H, H × L, and L × L series the non-Bare flies were heterozygous for different combinations of "high" and "low" chromosomes. Further explanation in text

Cross	t°C	Yeast	Flies counted	Percent non-Bare	x^2	df	*P*
H	25	F	11,203	32.89	20.90	9	0.01
L	25	F	9,084	27.93	92.57	8	<0.001
H × H	25	F	12,662	33.60	14.87	9	0.09
H × L	25	F	12,413	33.67	4.04	8	0.85
L × L	25	F	10,762	33.60	9.53	7	0.22
H	16	F	16,805	33.28	11.50	9	0.25
L	16	F	13,319	33.13	13.96	8	0.08
H × H	16	F	18,151	33.70	21.43	9	0.01
H × L	16	F	16,009	33.54	10.45	8	0.22
L × L	16	F	13,450	33.69	3.44	7	0.85
H	27	F	5,712	25.44	46.60	9	<0.001
L	27	F	5,052	19.70	222.03	8	<0.001
H × H	27	F	6,144	32.68	15.31	9	0.08
H × L	27	F	5,053	32.83	6.72	8	0.56
L × L	27	F	6,174	31.32	5.95	7	0.52
H	4	F	10,185	33.24	14.86	9	0.09
L	4	F	9,111	31.92	13.88	8	0.08
H × H	4	F	10,389	38.10	13.25	9	0.15
H × L	4	F	9,402	36.94	21.55	8	0.006
L × L	4	F	7,977	37.07	7.02	7	0.42
H	25	K	15,874	31.87	39.51	9	<0.001
L	25	K	15,293	29.94	36.94	8	<0.001
H × H	25	K	16,380	34.49	33.70	9	<0.001
H × L	25	K	13,567	34.45	28.54	8	<0.001
L × L	25	K	12,824	33.92	11.57	7	0.12
H	16	K	19,408	32.41	17.88	9	0.04
L	16	K	17,515	32.71	31.18	8	<0.001
H × H	16	K	19,857	32.66	18.61	9	0.03
H × L	16	K	17,323	33.52	12.29	8	0.14
L × L	16	K	15,944	34.01	44.39	7	<0.001
H	27	K	4,558	29.22	45.90	9	<0.001
L	27	K	3,893	28.41	16.63	8	0.04
H × H	27	K	5,598	34.30	18.08	9	0.03
H × L	27	K	5,823	34.79	5.11	8	0.75
L × L	27	K	6,196	32.44	15.36	7	0.03
H	25	Z	12,593	31.33	15.06	9	0.09
L	25	Z	10,322	26.43	165.78	8	<0.001
H × H	25	Z	11,313	33.58	11.99	9	0.20
H × L	25	Z	10,281	33.42	9.51	8	0.30
L × L	25	Z	11,568	32.57	14.89	7	0.04
H	27	Z	5,978	29.49	16.64	9	0.06
L	27	Z	5,480	25.20	244.48	8	<0.001
H × H	27	Z	7,922	36.23	32.46	9	<0.001
H × L	27	Z	6,987	35.69	18.78	8	0.02
L × L	27	Z	9,339	34.87	15.65	7	0.03

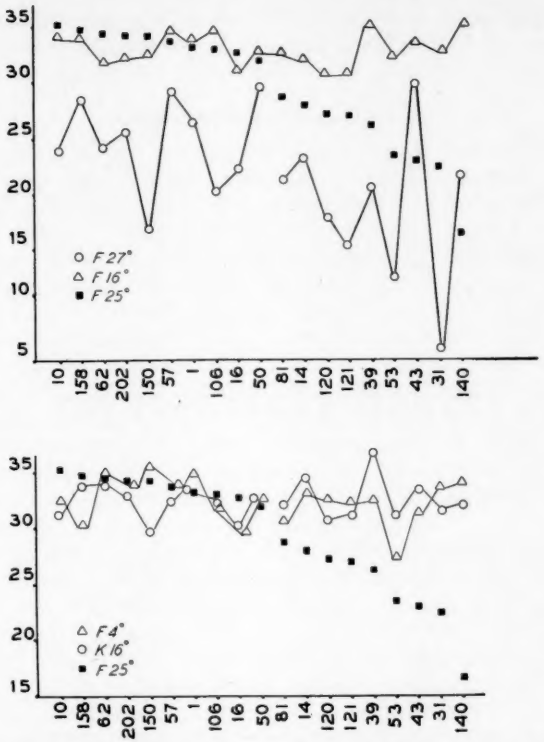

FIGURE 1.—Percentage of non-Bare flies emerging in various environments for homozygotes indicated by numbers (see text). Black squares indicate "standard" environment where homozygotes 10–50 are "high" and 18–140 are "low".

In cultures fed on Fleischmann's yeast but kept at 16° or at the variable low temperature (F 16° and F 4° in fig. 1 and table 4) the performance of the chromosomes is radically altered. In the first place, the homozygotes for H chromosomes are now, on the average, almost equal in viability to the homozygotes for L chromosomes (33.28% and 33.13% respectively at 16°, and 33.24% and 31.92% respectively at 4°, table 4). In other words, the average differences between the homozygotes which were supervital and subvital at 25° have largely disappeared at the lower temperatures. In fact, some of the highest proportions of non-Bare flies have been observed in cultures involving L chromosomes (chromosomes Nos. 39 and 140 at 16° and No. 140 at 4°, see fig. 1). It is interesting to note that the homozygotes for the chromosome No. 140 had the lowest viability at 25° (table 3 and fig. 1). Furthermore, at 16° the average viability of the homozygotes is only slightly lower (33.28% and 33.13%) than that of the heterozygotes (33.54% to 33.70%, table 4). At the variable low temperature

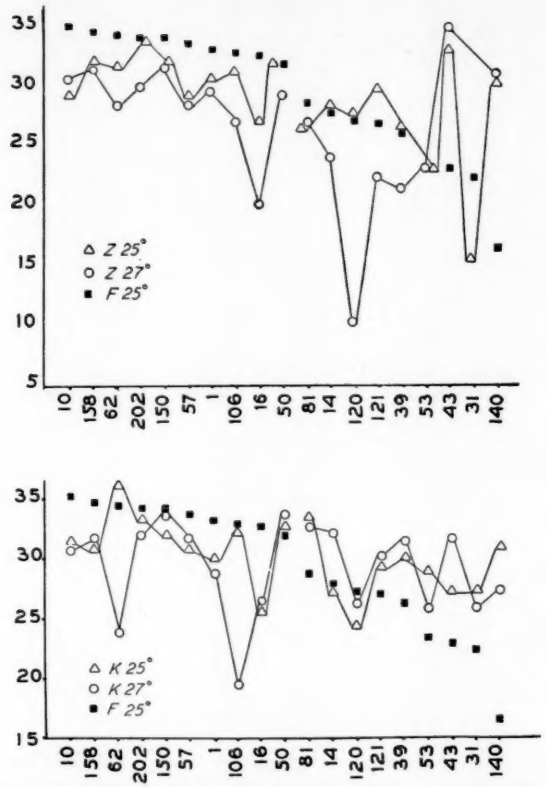

FIGURE 2.—Percentage of non-Bare flies emerging in various environments for homozygotes indicated by numbers (see text). Black squares indicate "standard" environment where homozygotes 10–50 are "high" and 18–140 are "low".

(4°, table 4) this is, however, not the case (31.92% and 33.24% for the homozygotes, 36.94% to 38.10% for the heterozygotes). It will be shown below that the homozygotes tend to be less fit than the heterozygotes in variable environments.

At high temperature and on Fleischmann's yeast (F 27° in fig. 1 and table 4) the situation changes again. The chromosomes which had similar records at 25° may behave quite differently at 27°, and vice versa. Thus, chromosome No. 43 produced semilethal homozygotes at 25°, but at 27° these homozygotes are as viable as any in either the "low" or the "high" groups. Conversely, the homozygotes for chromosome No. 31 are about as viable as No. 43 at 25°, but almost completely lethal at 27°. Chromosome No. 150 produced normally viable homozygotes at 25° but it is semilethal at 27°. The viability records of the different chromosomes at 27° are highly heterogeneous, as shown by the large chi-squares in the H 27 F and L 27 F lines in

table 4. The only respect in which the viability records at 25° and 27° are still consistent is that the mean viability of the homozygotes for H chromosomes is higher than that for L chromosomes (25.44% and 19.70%, table 4).

Influence of nutrition on homozygotes

Changing the nutritional variable without changing the temperature also causes alterations in the viability of the homozygotes. At 25° in cultures fed on Kloeckeraspora (K 25° in fig. 2 and table 4) some chromosomes improve their viability records (Nos. 62, 81, 121, 39, 53, 43, 31 and 140), while others change for the worse (Nos. 10, 158, 150, 57, 1, 16, 120). In general, the H chromosomes are more often the losers and L the gainers; in other words, the homozygotes which are supervital on Fleischmann's yeast are mostly subvital on Kloeckeraspora, while those subvital on Fleischmann's may gain on Kloeckeraspora, although not to the extent of becoming supervitals. The mean for H chromosomes (31.87%) is nevertheless higher than that for L chromosomes (29.94%, table 4). Comparison of the cultures fed on Fleischmann's with those fed on Zygosaccharomyces at 25° (Z 25°, fig. 2 and table 4) reveals a story similar to that in Kloeckeraspora cultures. The homozygote for chromosome No. 43 shows a spectacular improvement and is normal or even supervital on Zygosaccharomyces. A great improvement is also observed with No. 30, which is semilethal on Fleischmann's but normally viable on Zygosaccharomyces.

At 27°C and with Zygosaccharomyces food (Z 27° in fig. 2 and table 4) the viability of all homozygotes for H chromosomes is reduced; most of the L chromosomes also lose, but Nos. 43 and 140 gain very strikingly. The mean for H chromosomes is higher (29.49%) than for L (25.20%). The Kloeckeraspora-fed cultures at 27° (K 27° in fig. 1 and table 4) behave somewhat differently. Compared to the Fleischmann's fed cultures, the homozygotes for H chromosomes become more or less strongly reduced in viability (except Nos. 150 and 50), but the L chromosomes are mostly improved (except No. 81). The result is that the average for H chromosomes (29.22%) is not significantly higher than for L chromosomes (28.41%).

At 16° and with Klockeraspora-fed cultures (K 16° in fig. 1 and table 4) the viabilities of the homozygotes for H and L chromosomes become alike on the average (32.41% and 32.71% respectively). This does not mean, however, that the viabilities of all homozygotes have become uniform; indeed, the chi-square shows that a very significant heterogeneity continues to exist, at least among H chromosomes.

Comparing homozygotes with heterozygotes

At 25° and in cultures fed on Fleischmann's yeast, the homozygotes for H chromosomes are, on the average, about as viable as the heterozygotes, while the L homozygotes are less viable (table 4). This means, of course, only that the chromosomes were selected to produce just these effects, and that they continue to produce them on re-testing.

A much more significant result is that, in F 27°, F 4°, K 25°, K 27°, Z 25°, and Z 27° cultures, the mean viability of the H homozygotes is significantly lower than that of the heterozygotes in the same environment. This is less strikingly apparent

TABLE 5

Chi-squares for heterogeneity, comparing the viabilities of the heterozygotes carrying two subvital (L × L), one subvital and one supervital (L × H), and two supervital (H × H) chromosomes. All chi-squares have two degrees of freedom

Environment	Chi-square	Probability
F 25°C	0.03	0.98
F 4°C	3.00	0.22
F 16°C	1.32	0.50
F 27°C	3.77	0.15
K 25°C	1.20	0.50
K 16°C	7.53	0.02
K 27°C	8.29	0.015
Z 25°C	2.99	0.22
Z 27°C	3.88	0.15

in F 16° and K 16° cultures, but in these cultures the viabilities of all classes have become relatively uniform. The conclusion which follows is this: When a homozygote possesses a normal or a superior-to-normal viability, it does so only in a narrow range of environments.

Recessivity of subvital effects

The problem which interests us most is whether chromosomes which produce subvital homozygotes produce, on the average, less fit heterozygotes than do chromosomes which give normally viable or supervital heterozygotes. Table 4 contains data bearing on this problem. In every environment tried, we tested ten H × H heterozygotes, nine H × L heterozygotes, and eight L × L heterozygotes. Table 5 reports the chi-squares for heterogeneity for each of the nine environments. Each chi-square has two degrees of freedom. Only two of the nine chi-squares are significant between 1% and 2% levels. These are for cultures kept at 16° and 27° and fed on Kloeckeraspora. Now, referring back to table 4, one can see that in the K 27° series the highest viability average is for the H × L cultures, followed by H × H, and the lowest for L × L. This might suggest that chromosomes which are subvital in homozygous condition are subvital also when heterozygous. This is, however, invalidated by the K 16° series, in which the L × L average is the highest, followed by H × L and finally by H × H. This is the reverse of what would be expected if the subvitals were incompletely recessive.

The conclusion that the effects of the chromosomes when homozygous and when heterozygous are not correlated, can be tested further. Indeed, we have seen in figures 1 and 2 that the effects of a chromosome when homozygous are strongly modifiable by environmental changes. The average differences between chromosomes selected in one environment for supervital and subvital effects, tend to be more or less strongly reduced when the homozygotes are tested in other environments. For this reason, we have, in accordance with the suggestion of our colleague, PROFESSOR HOWARD LEVENE, computed rank order correlations for the effects of L chromosomes when homozygous and when heterozygous in a given environment. The results are shown in table 6.

TABLE 6

Rank order correlations between the effects of subvital chromosomes in homozygous and in heterozygous condition

Environment	Low × high	Low × low
F 25°C	+0.03	+0.24
F 4°C	−0.53	−0.52
F 16°C	+0.08	−0.43
F 27°C	−0.53	−0.83
K 25°C	+0.52	−0.21
K 16°C	+0.67	+0.24
K 27°C	+0.12	−0.40
Z 25°C	+0.43	−0.12
Z 27°C	−0.10	+0.55

Of the 18 rank order correlation coefficients in table 6, exactly one half (9) are positive and the other half are negative. Now, a significant positive correlation would indicate that, for each environment, a chromosome which is favorable in homozygotes is favorable also in heterozygotes, and vice versa. A significant negative correlation would indicate an opposite conclusion. In reality, only one of the positive correlations (+0.67, for K 16°) is significant at about the 5% level, and the same is true for one negative correlation (−0.83, for F 27°). The algebraic sum of all the correlations in table 6 is -0.79 ± 1.55, which is clearly not significant. We may conclude that no correlation between the effects of a chromosome when homozygous and when heterozygous is apparent in our data.

Heterogeneity of heterozygotes

From the absence of a correlation between the viability effects of chromosomes in homozygotes and heterozygotes, it does not follow that the heterozygotes are simply "normal" flies which are all more or less alike. They are not. Table 4 supplies the evidence that of the 27 classes of heterozygotes (3 groups, each tested in 9 different environments), only 13 do not show evidence of heterogeneity (probabilities 0.1 or higher). Among the remainder, 2 correspond to probabilities between 0.05 and 0.10, 6 between 0.01 and 0.05, and 6 to probabilities below 0.01. The conclusion may also be re-stated as follows: The viability of a heterozygote in a given environment depends on the nature of the chromosomes (viz., gene complexes) which it carries, but not on the effects which these chromosomes would have in double dose.

A word of caution must be added at this point. Examination of table 4 shows that the percentages of non-Bare flies, in cultures producing heterozygotes, were lower in some environments (F 27°) than in other environments (F 4°, Z 27°). This does not necessarily mean that all the chromosome combinations studied do better or worse respectively in these environments. Indeed, all the cultures produced wild type (= non-Bare) flies and flies which carried a chromosome containing a dominant mutant gene Bare, which does have some effects on the viability of its carriers (table 1). These effects are, naturally, subject to modification by the environments used in our experiments.

DISCUSSION

Except for dominant lethals, a certain time interval, which may embrace several or many generations, elapses between the origin of a deleterious mutant and its elimination from a Mendelian population. This interval is especially long for recessive mutants whose deleterious effects are sheltered from natural selection by being concealed in heterozygotes. The existence in Mendelian populations of a concealed store of deleterious variants is, then, simply a consequence of the less than perfect efficiency of natural selection. This is, however, only a part of the story, and, possibly, biologically the least meaningful part. Since the genotypes which are adaptively negative in some environments may prove positive in other environments, the concealed genetic variability may be regarded as a store of genetic raw materials (cf. DOBZHANSKY 1937; MATHER 1943; and other work). Owing to sexual reproduction, recombination of the genetic components of this store yields a large variety of genotypes, some of which fit the changing demands of the environment. This point of view remains valid, but it now appears that it is also an oversimplification.

Investigation of the inversions found in many species of Drosophila has revealed that the adaptedness of some natural populations rests to a considerable extent on a high incidence in these populations of heterotic inversion heterozygotes (cf., DOBZHANSKY 1951 for a review; also WALLACE 1954). It is formally correct, but certainly not very enlightening, to say that the low fitness of the inversion homozygotes is due to "deleterious recessives." Anyway, natural selection acts to perpetuate these recessives in the population rather than to eliminate them. One may also argue about whether a population consisting only of homozygotes would necessarily be ill-adapted (CAIN and SHEPPARD 1954), but it is a fact that natural selection establishes equilibrium frequencies of the inversions at which the mean adaptive value of the population as a whole is maximized. Now, the inversions proved to be only one of the mechanisms for maintaining balanced heterozygosis in populations. Recent work has shown that a considerable majority of the chromosomes in Drosophila populations are deleterious in homozygotes (WALLACE and MADDEN 1953; DOBZHANSKY and SPASSKY 1953, 1954). The fate of a chromosome under selection depends chiefly on the fitness of the gene complex it contains when it is in heterozygous combinations with other gene complexes which occur in the same Mendelian population; the fitness of the homozygotes is in general less important because of their rarity (WALLACE and KING 1952; WALLACE 1955; CORDEIRO and DOBZHANSKY 1954; and especially LERNER 1954). Some "deleterious recessives" are, thus, not fleeting but relatively permanent components of the gene pool of Mendelian populations. The data reported in the present article bear directly on this point: The heterozygotes which contain chromosomes which are subvital when homozygous have the same fitness as do heterozygotes for chromosomes which are normally viable or even supervital when homozygous.

CORDEIRO and DOBZHANSKY (1954) showed that different heterogeneous combinations, which carry pairs of chromosomes taken at random from the same population, vary in fitness in the same environment. The experiments described above confirm and extend this observation. "Normal" or "wild type" flies are an assem-

blage of many different genotypes, with different reaction norms and presumably also different ecological optima. In a way, this proves for Drosophila only what is so obvious in human populations, in which no two individuals are alike and often show their unlikeness by different reactions to the environment. But it is not obvious, and would be hard to foresee on theoretical grounds that the great variety of the reaction norms is an outcome of different combining ability of gene complexes which are mostly deleterious when homozygous. The load of mutations which a population carries is a dead weight only insofar as these mutations are deleterious both in homozygous and heterozygous condition, and in combination with most other genetic variants in the gene pool of the population. There is at least presumptive evidence that this is the case for many, though not for all, major mutations. We do not yet know how often the minor mutational changes are thus unconditionally harmful. But it is now apparent that the adaptive "norm" of a species established by natural selection is not genetically monolithic; it encompasses a large diversity of genetic elements, many of which show unexpected and unwelcome properties when homozygous.

SUMMARY

The effects in homozygous condition of 178 wild second chromosomes from a Texas population were studied at 25°C and in cultures fed on Fleischmann's yeast (*Saccharomyces cerevisiae*). From this sample, 10 chromosomes were selected which produced normally viable or supervital homozygotes (these chromosomes are denoted H), and 9 chromosomes which produced subvital homozygotes (these chromosomes are denoted L). The effects of these chromosomes when homozygous, and also when heterozygous in H × H, H × L, and L × L combinations, were studied at 4 different temperatures and in cultures fed on 3 different yeasts. The viability of the homozygotes proved to be highly sensitive to environmental modification (see figs. 1 and 2, and table 4). The viability of the heterozygotes is relatively more uniform and stable; nevertheless, the different heterozygotes are not all alike. It may be inferred that each heterozygous combination of chromosomes has a reaction norm somewhat different from other combinations. The important point is, however, that the heterozygotes which carry chromosomes which are subvital when homozygous, are not systematically inferior in fitness to those carrying chromosomes which are normally viable or supervital when homozygous. In this sense, the subvitality of most chromosomes found in natural populations, may be said to be completely recessive. However, since the heterozygous combinations are diversified, the chromosomes, or more precisely the gene complexes which they contain, do vary in their effects in heterozygotes. The subvitality in homozygous condition of most chromosomes found in a Mendelian population is a consequence of the gene pool being organized by natural selection to function as a coadapted system of numerous genetic variants.

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APPENDIX

Genetic variability of marginal populations

An hypothesis has been put forward that the amount of adaptive polymorphism present in a Mendelian population is positively correlated with the diversity of ecological niches which this population has mastered (DA CUNHA, BURLA, and DOBZHANSKY 1950). One of the tests of the validity of this hypothesis has been made by TOWNSEND (1952). He found that populations of *D. willistoni* which inhabit the margins of the distribution area of the species (Florida, Cuba) contain fewer chromosomal inversions than do populations of the central portion of the distribution area (central Brazil). The incidence of chromosomes which were lethal when homozygous was, however, only slightly lower in Florida than in central Brazil. Low frequencies of lethal chromosomes have, however, been recorded by CORDEIRO (unpublished) in certain populations from southern Brazil, near the southern margin of the distribution of *D. willistoni*. The population of *D. pseudoobscura* which has yielded the chromosomes used in the investigation described in the present article is a marginal one, since this species is rare or absent to the east of Austin, Texas (PROF. J. T. PATTERSON personal communication). The data in tables 1 and 2 permit the making of certain comparisons with other populations of the same species.

The frequency of lethal and semilethal second chromosomes in the Texas population is 31.5 ± 3.5% of the total (see above). This agrees very well with the figure for the populations of the Yosemite region of California, which is 33.0 ± 4.5%, but is higher than that for San Jacinto population, which is 21.3 ± 1.8% (see table 6, DOBZHANSKY and SPASSKY 1954). The California populations are not marginal. There is no evidence in our data that the marginal population in Texas has fewer lethals than do at least some non-marginal populations.

Another comparison is more instructive. Consider the 122 quasi-normal chromosomes listed in table 2. The mean frequency of non-Bare flies produced in cultures involving these chromosomes turns out to be 30.66 ± 0.38%. This can be compared with the mean frequency of 34.20 ± 0.26% in control cultures (table 1). Dividing the two figures we conclude that the mean viability of homozygotes for quasi-normal chromosomes from the Texas populations is 89.65 ± 1.11% of normal viability. The corresponding figure for Yosemite populations is 75.00 ± 1.22% (table 7 in DOBZHANSKY and SPASSKY 1954). The difference is clearly significant.

The data on the San Jacinto populations are, unfortunately, not quite comparable. The problem of genetic variability in marginal populations of a species is wide open. It seems possible that different classes of genetic variants do not behave in the same way. A marginal population may contain a more limited diversity of variants which serve as materials for construction of balanced polymorphic systems than a central population. Lethals and other drastic genetic changes may obey different rules.

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GENETICS OF NATURAL POPULATIONS. XXIV. DEVELOPMENTAL HOMEOSTASIS IN NATURAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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IN his important book on *Genetic Homeostasis*, LERNER (1954) concludes "that heterozygosity has a dual function in the life of Mendelian populations. On the one hand, it provides a mechanism for maintaining genetic reserves and potential plasticity, and on the other, it permits a large proportion of individuals to exhibit combinations of phenotypic properties near the optimum. Underlying both processes is the superior buffering ability of heterozygotes as compared with homozygotes." The foregoing article of DOBZHANSKY, PAVLOVSKY, SPASSKY and SPASSKY (1955) describes the heterozygosity in the second chromosomes of a natural population of *Drosophila pseudoobscura*. The purpose of the present article is to examine the buffering ability of the heterozygotes and homozygotes for some of these chromosomes. To this end, "The ideal test would consist of comparisons of environmental variability in fitness of these types" (LERNER 1954). Our material permits an approach to this "ideal test", although the data obtained so far are limited to examination of only the viability between the egg and adult stages of the carriers of certain heterozygous and homozygous genotypes under a relatively limited variety of environmental conditions. The viability is, of course, a component of fitness but is not synonymous with it.

THE DATA

The nature of the material used and the experimental techniques employed are described in the companion article of DOBZHANSKY, PAVLOVSKY, SPASSKY and SPASSKY. The essentials relevant to our present purposes are as follows. Nineteen second chromosomes were isolated from a natural population of *Drosophila pseudoobscura*. Ten of these, noted in the tables below by H ("High") produced normally viable or supervital homozygotes in cultures raised at 25°C and fed on Fleischmann's yeast (F). Nine chromosomes, denoted by an L ("Low"), produced subvital homozygotes under the same conditions. The viability of these nineteen homozygotes was then tested in nine different environments. These environments involved four different temperatures—27°, 25°, 16°C, and a variable temperature regime ranging from 4° to 25°C, and feeding on three different yeasts, namely Fleischmann's *Saccharomyces cerevisiae* (F), a species of Klockeraspora (K), and of Zygosaccharomyces (Z). Twenty-seven crosses were made in which heterozygotes for different combinations of H and L chromosomes were obtained (see table 2).

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All tests of the homo- and heterozygotes in a given environment were carried out simultaneously; the experiments with the F yeast were done somewhat earlier than those with K and Z yeasts.

Six replicate cultures containing the offspring of the same group of parents were made to test the viability of every homozygote and of every heterozygous combination of chromosomes. Counts of the flies with and without the marking mutant gene (Bare) were made in all cultures. The primary data consist, then, of records of the numbers of Bare and non-Bare flies in the different cultures. The frequencies of the non-Bare flies are expressed in percentages of the totals. The statistics discussed below are derived from these percentages.

ANALYSIS OF VARIANCE

The body of data at our disposal furnishes three kinds of quantities for analysis. (1) The first and most basic of these is the average viability of the homozygotes and heterozygotes for certain chromosomes, expressed as percentages of non-Bare (wild type) flies. The values of these viabilities are given in table 4 of the preceding paper (DOBZHANSKY, PAVLOVSKY, SPASSKY and SPASSKY 1955). (2) Quantities which we will call microenvironmental variances. The replicate cultures were grown under conditions which were kept as nearly uniform as practicable in large-scale experiments. Nevertheless, the conditions did vary because of the different amounts of food in the different culture bottles, different degrees of crowding, as well as minor differences in temperature, microorganisms in the food, etc. Accordingly the observed variance between replicate cultures consists of two distinct components. One is the ordinary binomial sampling variance, the other the variance in viability due to the differences between replicate cultures. By subtracting the calculated binomial sampling variance from the observed variance we obtain an estimate of the second component, which we call microenvironmental variance. If p_i is the observed percentage of wild type and n_i the total number of flies in the ith culture, and c is the number of cultures, we then let $\overline{p} = (\sum p_i)/c$ be the unweighted mean viability, and then estimate the binomial sampling variance as $\sigma_b^2 = (\sum \frac{\overline{p}(100 - \overline{p})}{n_i})/c$ (See the similar computation in WALLACE and MADDEN 1953). The means of microenvironmental variances for all genotypes of a given kind raised at a given temperature on a given yeast are given in table 1. (3) The third kind of quantity we will consider is the macroenvironmental variance; i.e. the variance of the survival rates of a given genotype over the different macroenvironments or yeast-temperature combinations. These are obtained in a similar way to the microenvironmental variances. We let p_i and n_i now be the percentage of wild type and number of flies in the combined cultures of a particular macroenvironment, and subtract σ_b^2, defined as above, from the observed variance of the viability of that genotype over all nine macroenvironments. The resulting quantity will be called the macroenvironmental variance. Values of these variances for each of the genotypes in this study are given in table 2.

Each of the three types of quantity discussed above adds something to our understanding of the situation, and they will be analyzed separately. We first consider

TABLE 1

Microenvironmental variance between replicate cultures raised under different conditions. L—chromosomes which are subvital, and H—chromosomes which are supervital when homozygous. F—Fleischmann's yeast, K—Kloeckeraspora yeast, Z—Zygosaccharomyces yeast

Environment	Homozygotes L	Homozygotes H	Heterozygotes L × L	Heterozygotes L × H	Heterozygotes H × H
F 25°C	19.98	8.58	−0.84	3.57	3.52
F 27°	6.06	37.63	−6.46	4.87	−3.56
F 16°	12.50	6.66	−3.39	3.82	−2.64
F 4°	5.69	3.98	10.23	−5.02	1.50
K 25°	18.06	3.88	8.17	7.41	5.74
K 27°	36.14	13.80	2.84	2.49	10.18
K 16°	4.87	5.12	2.91	4.15	6.42
Z 25°	47.00	11.62	0.96	5.36	18.51
Z 27°	13.52	33.02	17.29	18.36	9.44
Mean	18.20	13.81	3.53	5.00	5.46
Std. Error	±3.88	±1.96	±1.45	±1.59	±1.89

the analysis of the basic data, the viabilities per se. To start with, we restrict ourselves to a particular kind of cross, such as low homozygotes or low × low heterozygotes. The data then fall into a two way analysis of variance with replications. One criterion of classification is genotype: the particular chromosome or combination of chromosomes involved. The other criterion is the nine macroenvironments formed by the combinations of yeast and temperature tested. For each combination of genotype and environment there are six viability values from the replicate cultures. A standard analysis of variance is then performed and the effects of genotype, environment and genotype-environment interaction are tested. A significant effect of genotype implies that different genotypes have different mean viabilities when averaged over all nine environments. Similarly, a significant effect of environment means that the mean viability of all the genotypes of a certain kind varies from environment to environment. Finally, a significant F statistic for interaction implies that in addition to any possible average effects of genotype and environment, there are also unpredictable results for specific genotype-environment combinations. Mathematically, if there were no interaction, the true viability of a particular genotype-environment combination would be equal to the grand mean of all combinations, plus the difference between the average viability of that genotype and the grand mean, plus the difference between the average viability in that environment and the grand mean; and the observed viability would differ from this only because of the random variability between replicate cultures. A simple numerical example may clarify the idea of interaction. Let the grand mean be 33, and let genotype I be one less on the average and genotype II one more, while environment A is 2 less on the average and environment B, 2 more. Then the predicted values without interaction would be IA = 30, IB = 34, IIA = 32, IIB = 36, while a possible set of values with interaction would be IA = 31, IB = 33, IIA = 31, IIB = 37. In the first case the difference of I and II is 2 in environment A and 2

TABLE 2

Macroenvironmental variance between series of cultures raised in different environments. L—chromosomes which are subvital, and H—chromosomes which are supervital when tested in homozygous condition on Fleischmann's yeast at 25° C

Homozygotes		Heterozygotes	
Chromosome no.	Variance	Chromosome combination	Variance
L 14	12.23	L 14 × L 31	1.11
L 31	156.42	L 31 × L 39	8.32
L 39	8.22	L 39 × L 43	6.31
L 43	2.09	L 43 × L 53	3.28
L 53	31.78	L 81 × L 120	7.12
L 81	13.65	L 120 × L 121	5.62
L 120	48.46	L 121 × L 140	1.95
L 121	27.10	L 140 × L 14	1.20
L 140	36.61		
Mean L	37.40	Mean L × L	4.36
H 1	4.41	H 1 × L 14	2.69
H 10	1.67	H 10 × L 31	1.20
H 16	10.02	H 16 × L 39	2.98
H 50	0.23	H 50 × L 43	2.18
H 57	2.66	H 57 × L 53	4.01
H 62	10.87	H 106 × L 81	−0.63
H 106	27.08	H 150 × L 120	6.18
H 150	27.87	H 158 × L 121	−1.50
H 158	1.71	H 202 × L 140	1.77
H 202	4.85		
Mean H	9.14	Mean H × L	2.10
		H 1 × H 10	10.46
		H 10 × H 16	5.03
		H 16 × H 50	2.60
		H 50 × H 57	3.56
		H 57 × H 62	3.75
		H 62 × H 106	3.13
		H 106 × H 150	3.60
		H 150 × H 158	5.28
		H 158 × H 202	3.13
		H 202 × H 1	4.10
		Mean H × H	4.46

in environment B, while in the second this difference is 0 in environment A and 4 in environment B. Hence the departure of the viability of a genotype-environment combination from the predicted value is a measure of the interaction. In biological terms the interaction is a measure of the specific biological interaction of genotype with environment; e.g. a genotype which has a superior viability in some environments may be inferior in others.

TABLE 3

Analysis of variance of viabilities

	Genotypes			Environments			Interaction			Error	
	MS	df	*F*	MS	df	*F*	MS	df	*F*	MS	df
L	78.8	8	3.93***	219.4	8	10.95***	20.04	64	2.98***	6.717	405
H	21.85	9	3.56**	71.4	8	11.65***	6.13	71	1.066	5.749	445
L × L	5.46	7	1.65	22.45	8	6.78***	3.31	56	.918	3.605	360
L × H	5.62	8	1.70	16.37	8	4.96***	3.30	64	.754	4.376	405
H × H	13.12	9	3.13**	31.19	8	.45***	4.19	72	.956	4.379	450

** Significant at the 1% level.
*** Significant at the 0.1% level.

The analysis of variance is given in table 3. The error mean square is used to test the interactions, while the interaction mean squares are used for testing the main effects. This is done because the main effects are not biologically meaningful unless they are significantly larger than the interactions. The effect of macroenvironment is highly significant for all five types of cross, as might be expected, since these environments are extremely different. The effect of genotype is highly significant for low homozygotes, significant at the 1% level for high homozygotes and high by high heterozygotes, and not significant but possibly present for low by low and high by low heterozygotes. There is also a significant interaction for low homozygotes, but in the other four types of cross the variance between replicates explains all departures from the values predicted from the main effects.

The fact that only one significant interaction is found seems to contradict the findings in the companion paper on the unpredictability of the behavior of different genotypes in different environments. However, this was most striking for L homozygotes, where we *do* find significant interaction. The unpredictability in the H homozygotes, which were selected because they were supervital in one environment (F25), shows up mainly as a regression toward the expected viability for random homozygotes in other environments. Statistically, this appears as a main effect (lower average viability in the other environments), rather than as interaction, in the analysis of the H homozygotes. The behavior of the heterozygotes, on the other hand, is more predictable.

We now turn to the analysis of the microenvironmental variance. Here each microenvironmental variance is calculated from the six replicates, giving a single figure for each genotype-macroenvironment combination. Consequently there is no error mean square against which to test the interaction mean squares. Accordingly the interaction will not be tested. In the light of the comparative unimportance of interaction for the viability itself, this seems not too serious a loss.

The analysis of variance for the microenvironmental variances is given in table 4. While the effect of genotype and macroenvironment are both larger for homozygotes than for heterozygotes, the interaction, or error, is also larger, so that only the effect of environment is significant, and this only for high homozygotes. In addition the effect of macroenvironment is barely significant for low by low heterozygotes, but this could well be due to chance, since one *F* out of nine would often be sig-

TABLE 4

Analysis of variance for microenvironmental variances

	Genotypes			Environments			Interaction	
	MS	df	*F*	MS	df	*F*	MS	df
L	2050	8	1.68	1695	8	1.39	1223	64
H	475.9	9	1.40	1221.9	8	3.58**	340.8	71
L × L	245.9	7	1.61	430.1	8	2.82*	152.3	56
L × H	179.1	8	.88	332.4	8	1.62	204.7	64
H × H	164.7	9	.51	469.9	8	1.46	321.4	72

* Significant at the 5% level.
** Significant at the 1% level.

nificant at the 5% level. Even the result for high homozygotes appears upon inspection to be due solely to a high microenvironmental variance in one of the nine macroenvironments, namely, Fleischmann's yeast at 27°. There is thus no evidence for any consistent average effects of either genotype or environment on microenvironmental variance.

No analysis of variance is possible for macroenvironmental variance for a given type of cross, since there is only one value for each genotype. Formally, it would be possible to use a test for homogeneity of variances, but for various technical reasons such a test was not felt to be valid for this data.

We turn now to comparisons between different types of cross. The mean viabilities, with their standard errors, for each type of cross as given in the companion paper, are as follows: L, 27.91 ± 0.99; H, 30.68 ± 0.49; L × L, 33.56 ± 0.28; L × H, 34.31 ± 0.26, H × H, 34.4 ± 0.38. In each case the standard error is the square root of the mean square for genotypes divided by the number of genotype-environment combinations. The reason for this is that we desire to draw general conclusions about the types of crosses, and the mean viability of "low" homozygotes, for example, would have been quite different if some other set of nine chromosomes had been used. A measure of the expected difference due to this cause is given precisely by the mean square for genotypes. The actual viabilities given above are in precisely the order one might expect, with low homozygotes the poorest and high by high heterozygotes the best. However, the differences between the three classes of heterozygotes are not significant. The difference between low homozygotes and high homozygotes is significant at the 5% level, while the difference between homozygotes and heterozygotes is significant at the 0.1% level, giving clear evidence of heterosis.

A similar analysis was made for microenvironmental variance. In this case the standard error was calculated from the interaction mean square, since none of the effects of genotype was significant, and the interaction afforded more degrees of freedom, permitting the use of tables of the normal distribution rather than STUDENT'S *t*. The mean values and their standard errors are given in table 1. The microenvironmental variance is greater for low than for high homozygotes but not significantly so. Homozygotes are very significantly higher than heterozygotes (*P* less than 0.1%), and there are no significant differences between classes of heterozygotes.

The analysis of the figures for macroenvironmental variance in table 3 must be done in a slightly different way. Homeostasis resulting from heterozygosity would be evidenced by fairly uniformly low macroenvironmental variance for heterozygotes, and by a higher value for at least some of the homozygotes, and this is what is observed. However, the variability of these values among homozygotes is so high as to attach a large standard error to their means, and this obscures the difference between the mean of homozygotes and the mean of heterozygotes. Since the larger standard error is itself evidence of a difference between homozygotes and heterozygotes, it is valid to compare the difference of the means with the error calculated from heterozygotes alone. Accordingly, the pooled sum of squares for heterozygotes was divided by its degrees of freedom, and then multiplied by the sum of the reciprocals of the number of homozygotes and number of heterozygotes, and used as the variance of the difference. Similarly the sum of squares for high homozygotes was used to test the low versus high homozygote comparison. The result is similar to those before. There is no significant difference between heterozygotes. The difference between homozygotes and heterozygotes is highly significant ($t = 6$), and the difference between low and high homozygotes also gives $t = 6$. The macroenvironmental variance for L31, 156.42, far exceeds all the others. However, even if it is excluded, the mean for lows (22.3) is significantly higher than that for highs.

Finally, a remarkable fact should be noted. The actual values of macro- and microenvironmental variances averaged over all experiments for a given type of cross are closely comparable. In other words the "minor" fluctuations in environment between replicate cultures seem to have as great an effect on viability as the major differences in yeast and temperature. This point seems worthy of further study.

VARIANCE AND VIABILITY

The broad outline of the situation is now clear. The overall viability of homozygotes is, in most environments, lower than that of heterozygotes. The viability of homozygotes is also very sensitive to both micro- and macroenvironmental differences, while the viability of heterozygotes is relatively stable. Since the viability of heterozygotes tends to be uniformly high, and that of homozygotes tends to be frequently but not uniformly low, the viability and sensitivity to environmental changes are really two sides of the same coin. It should be kept in mind that some chromosomes (H) were chosen because they produced in a certain environment highly viable homozygotes, and other chromosomes (L) because they gave subvital homozygotes. When the environment was varied, the H homozygotes remained on the average more viable than the L homozygotes, though with many exceptions. The H homozygotes showed also lower variances than did L homozygotes (tables 1 and 2). On the other hand, the L homozygotes show a significant interaction of viability in the different environments, and both classes of homozygotes show differences among themselves in average viability in different environments. The heterozygotes are more uniform. There are no differences between H × H, H × L, and L × L heterozygotes, and no average differences or interactions with the environment for different heterozygous combinations.

The relationship we have repeatedly observed between low viability and high environmental variability is so important that it is desirable to study it more directly.

Accordingly a scatter diagram was prepared, plotting the macroenvironmental variances for the various low and high homozygotes against the corresponding mean viabilities. Homozygotes for chromosome L31 had by far the largest variance and smallest viability. Even after deleting this point the remaining points showed considerable correlation, giving $r = -0.78$, which for 16 degrees of freedom is significant at the 0.1% level, giving a 95% confidence interval of -0.45 to -0.9. The correlation of the same mean viabilities with the corresponding microenvironmental variances was -0.57, which is significant at the 2% level, giving a 95% confidence interval of -0.1 to -0.8. The corresponding correlation of micro- and macroenvironmental variances was $+0.41$, which is not significantly different from 0. Calculation of the corresponding partial correlations gives slightly smaller values for the first two, but a value of only -0.02 for the partial correlation of micro- and macroenvironmental variances after removing the effect of viability. Evidently then, the chromosomes with low mean viability are usually the ones which are poorly adapted to some but not all of the tested macroenvironments and therefore show large variability. To a lesser extent such chromosomes also react erratically to the smaller environmental differences between replicate cultures. On the other hand there seems to be no interrelationship between macro- and microenvironmental variability except to the extent that both tend to be associated with low mean viability.

Looking at the data differently, and using the unweighted average values of all low and high homozygotes in each macroenvironment, the correlation between viability and microenvironmental variance is -0.34, giving a 95% confidence interval of -0.7 to $+0.2$. There is thus indication that less favorable macroenvironments may produce greater microenvironmental variances. Finally separate scatter diagrams were made for L and H, plotting microenvironmental variances against the corresponding viabilities for each genotype-environment combinations. The result for L's was a widely scattered constellation of points showing no apparent correlation. A statistical test confirmed the absence of any correlation. The result for H's was similar, but with some slight appearance of negative correlation that was barely significant at the 5% level. The difference from the previous results is due to the facts that only microenvironmental variances are available here, the regularizing effect of averaging is missing, and the larger number of experimental values permitted separating the L from the H homozygotes.

Finally it should be said that there is no evidence of any correlation between viability and variances for the heterozygotes. This is not surprising since the observed viability range for the heterozygotes is very much smaller than for homozygotes, and the number of different heterozygotes studied is limited (27).

DISCUSSION

Some analogies of our results with those observed in maize are worth mentioning. Differences we observe between viability of different hybrids are due to what maize geneticists call "Total combining ability" of the parent homozygotes. The present data is not well suited to separating general and specific combining ability. In maize such characters as yield in F_1 hybrids of two inbred strains show a low correlation

with the corresponding character of the two parent inbred strains (see e. g. the review by SPRAGUE 1946). We might expect less correlation for viability, since natural selection will reduce the additive genetic variance or heritability for this character. ROJAS and SPRAGUE (1952) have studied the interactions of genotype with year and locality for F_1 hybrids in maize and find these components of variance to be of the same order of magnitude as the main effects of genotype, but much smaller than the main effects of year and locality. In our data, on the other hand, for the most part neither main effects of genotype nor genotype-environment interactions are significant for heterozygotes. Again this is probably due to greater selection for the character, viability, that we are studying.

In recent years, several investigators have, largely independently, arrived at the conclusion that heterosis, the high fitness of heterozygotes relative to homozygotes, is often due to the superior buffering abilities met with in many heterozygotes. ROBERTSON and REEVE (1952) noted that the environmental variance of the wing length is greater in inbred lines of *Drosophila melanogaster* than in F_1 hybrids between these lines. They suggested that the decrease of the variance following hybridization may be due to greater biochemical versatility of the heterozygotes. The heterokaryons of Neurospora studied by EMERSON (1952) and others may be regarded as models showing how such a biochemical versatility could arise. DOBZHANSKY and WALLACE (1953) compared the survival rates in replicate cultures of homozygotes and heterozygotes for certain chromosomes from natural populations of *Drosophila pseudoobscura*, *D. persimilis*, and *D. prosaltans*, and from experimental populations of *D. melanogaster*. The survival rates were heterogeneous in homozygotes more often than in heterozygotes in all four species. The mean viability of the homozygotes was decidedly lower than that of the heterozygotes. Therefore it could be inferred that the developmental paths of most heterozygotes are better buffered against environmental disturbances, are more homeostatic, than the developmental paths of most homozygotes. It was further surmised that the superior homeostatic properties of the heterozygotes are a consequence of coadaptation of the gene contents of the chromosomes composing the gene pool of a Mendelian population. The coadaptation is the outcome of natural selection.

MATHER (1953) found that the numbers of sternopleural bristles on the two sides of the body in the same individual are more often different in inbred lines of *D. melanogaster* than in the F_1 and F_2 hybrids between these lines. MATHER's interpretation parallels rather closely that given by DOBZHANSKY and WALLACE (see above). except that MATHER speaks not of homeostasis but of "canalization", a term suggested by WADDINGTON (1942). LEWIS (1954) observed that, in a certain environment, the F_1 hybrids between two lines of tomatoes are less variable with respect to the flower number than are these lines themselves. However in a different environment the variability was uniform. LEWIS suggests an interesting model which relates the phenotypic stability of a trait with the degree of dominance in variable environments of the genes which influence that trait. The interesting experiments of TEBB and THODAY (1954) lead them to conclude that heterozygosity does not necessarily promote homeostasis and that coadaptation of gene alleles or gene complexes may be necessary to bring homeostasis about. On the other hand, LERNER (1954) has

critically reviewed the evidence concerning this homeostatic adjustment in homo- and heterozygotes. He is inclined to believe that, at least in domestic animals, "mere diversity of alleles at different loci may provide a sufficient basis for what is usually referred to as heterosis," although it is possible that "coadaptation of the genetic contents of homologous chromosomes plays a greater role in natural than in domestic populations."

The evidence presented above shows very clearly that the average environmental variance of the survival rates is greater in homozygotes than in heterozygotes. The heterozygotes are better buffered, both with respect to the microenvironmental and the macroenvironmental variations. This much is a confirmation of the results of DOBZHANSKY and WALLACE (1953). Now we can go somewhat further. In contrast to DOBZHANSKY and WALLACE, we have not worked with a random sample of chromosomes from a population. The H chromosomes were selected because they produced, in a certain macroenvironment, homozygotes which were about as viable as an average heterozygote. The L chromosomes produced, in the same environment, subvital homozygotes. The homozygotes for H chromosomes were, however, not as fit as the heterozygotes when the environment was altered. Not only is the mean viability of the homozygotes often below that of the heterozygotes (cf. table 4 in the companion paper by DOBZHANSKY, PAVLOVSKY, SPASSKY, and SPASSKY), but both the micro- and the macroenvironmental variances for H homozygotes are significantly greater on the average than those for the heterozygotes (see tables 1 and 2). In fact, the environmental variance for H homozygotes (13.81 ± 1.96) is not significantly smaller than that for L homozygotes (18.20 ± 3.88). We may conclude that the homozygotes for some of the chromosomes found in natural populations of Drosophila are "narrow specialists". Such homozygotes do quite well in a restricted range of environments, but they lack the resilience necessary to maintain their fitness in other environments. By contrast, the heterozygotes are more often many-sided and versatile in their adaptedness, hence able to live successfully in a broader range of environments.

The objection may be raised that it is high, rather than uniform, rate of survival in different environments that is necessary for adaptedness. A lethal which dies in all environments is surely not well adapted. But this is sheer confusion; high survival is correlated with versatility in a range of environments; homeostasis results in high as well as uniform survival rates. The survival rates of the heterozygotes, measured in percentages of non-Bare flies in the cultures, are high (table 4 in the companion paper) as well as relatively uniform (table 2 of the present paper). The variations in these percentages in different environments may be due as much to the environmental sensitivity of the Bare as of the non-Bare flies. Conversely, the homozygotes show lowered as well as variable survival rates. It should of course be realized that all the Bare flies contain heterozygous combinations of a Bare chromosome and one of the tested chromosomes, since homozygous Bare rarely survives, and that hence the Bare flies in our cultures may be expected to behave more like the heterozygotes than like homozygotes for chromosomes taken from nature.

To understand clearly the relation of homeostasis to adaptedness, it must be kept in mind that homeostasis does not imply a stationary state (WADDINGTON 1953) but

a dynamic stability. According to CANNON (1932), "In an open system, such as our bodies represent, compounded of unstable materials and subjected continually to disturbing conditions, constancy is in itself evidence that agencies are acting, or ready to act, to maintain this constancy." Action is, however, change; homeostasis is brought about by changes in some processes which result in stability of other processes. Among the vertebrates, the composition of the blood is maintained fairly constant; this constancy is due, however, to the activities of the kidneys which function differently under different circumstances. What is essential about homeostasis is that it enables life to continue despite the variations in the environment. Homeostatic adjustments permit the development, whether that of an embryo or that of an adult, to follow one of a limited variety of paths established in evolution under the control of natural selection. Straying away from these paths results in death (LERNER 1954, see particularly his fig. 6). On the other hand, homeostasis does not prevent the development from switching from one of these historically established paths to another established path. The ability of the organism to follow any one of these paths is, in fact, highly adaptive. Thus, on the human level, the plasticity of behavioral developmental patterns confers a very high adaptability on our species. What homeostasis does is to avoid the develcpmental processes being deflected in haphazard ways, resulting in non-adaptive modifications (morphoses according to SCHMALHAUSEN 1949).

The simplest form of homeostasis is exemplified by reversible physiological reactions which permit the functioning of the body and the organs to continue unimpeded. Kidney function, temperature regulation, and functional hypertrophy or atrophy of organs are good examples. By insensible gradations this *functional homeostasis* merges with physiological reactions which result in *developmental homeostasis*. Developmental homeostasis, which WADDINGTON (1942, 1953) prefers to call canalization, permits the development to keep to a definite path or to switch over to a closely related path. SCHMALHAUSEN'S (1949) physiogenic modification is also a related concept. A given repertory of functional and developmental homeostatic mechanisms is, of course, determined by the norm of reaction of each genotype. LERNER'S (1954) *genetic homeostasis* refers to self-regulatory properties not of individual organisms but of Mendelian populations. It may seem that genetic homeostasis is a phenomenon entirely separate from functional and developmental homeostasis. But LERNER argues, and we believe convincingly, that the genetic homeostasis is brought about by essentially the same mechanisms of heterozygosity and coadaptation of gene alleles and gene complexes as those which underly the other forms of homeostasis.

Surely, the relationships between the different forms of homeostasis, between homeostasis and heterosis, and between homeostasis and adaptedness are far from completely understood. The scene is too diverse and contradictory. The field is a new one, and more data are needed. It is particularly important to know to what extent these phenomena are due to what LERNER (1954) has called "mere diversity of alleles" and to what extent to previous selection and coadaptation of these alleles. LERNER'S summary of the pertinent evidence now available suggests rather that the situation may be different in organisms with different types of reproductive biology.

SUMMARY

The viability of homozygotes for 19 different second chromosomes, and of 27 different heterozygous combinations of the same chromosomes, has been studied. These homozygotes and heterozygotes have been exposed to 9 different "macroenvironments" (different temperatures and nutrient media) and to different "microenvironments" (environmental differences which occur in replicate cultures in the same macroenvironment). Homozygotes show greater mean macroenvironmental and microenvironmental variances of survival rate than do heterozygotes. The developmental patterns of the heterozygotes are more homeostatic, better buffered against environmental disturbances than those of the homozygotes. The viability and the homeostatic buffering are positively correlated, the viability and the environmental variances show a negative correlation. The relationships between viability, homeostasis, and variability are discussed.

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A GENETIC STUDY OF SEGREGATION IN A TRANSLOCATION HETEROZYGOTE IN DROSOPHILA[1]

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DURING meiosis, homologous chromosomes normally pair with each other and separate whereas the members of pairs of non-homologous chromosomes assort independently of one another. This behavior is disturbed, however, in individuals carrying a translocation. It is well known that at the first meiotic division separation of the four components of a translocation configuration may take place in a number of different ways and that each type of first division separation gives rise to two kinds of gametes, each the complement of the other. It is ordinarily expected that the two complementary products from any one type of separation will be produced equally frequently.

Certain cases in *Drosophila melanogaster* which did not meet the expectation of equal recovery were reported by GLASS (1934, 1935) who found that from crosses of females, heterozygous for a translocation of the type diagrammed in figure 1, by heterozygous translocation males, female gametes with 2S + 3′ were recovered twice as frequently as those with 2′L′ + 3 (see the legend to figure 1 for explanation of symbols). The results from other kinds of tests established beyond any doubt that this discrepancy could not be accounted for on the basis of a difference in the viability between these types.

NOVITSKI (1951) showed that from a Drosophila female carrying two homologous X-chromosomes differing in length, the shorter of the two is recovered about twice as often as the longer. This phenomenon of non-random disjunction depends upon the occurrence of an exchange between the two homologs, with the formation of a dyad composed of two structurally dissimilar chromatids. He suggested that the inequality of the frequencies of the two types of gametes from the translocation heterozygote described above might also be a manifestation of non-random disjunction. The experiments to be described below were designed to test the validity of this explanation for the unusual behavior of the translocation heterozygote, as well as to test certain other possibilities.

GENETIC CONSEQUENCES OF AN EXCHANGE IN THE INTERSTITIAL REGION

The assumption of non-random disjunction involves a consideration of the relationship between a given type of separation and the types and frequencies of resultant gametes arising from tetrads in which an exchange has taken place in the interstitial

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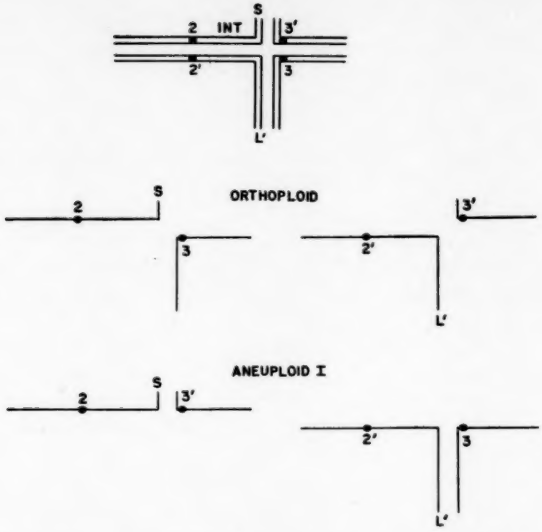

FIGURE 1.—The pairing configuration of a translocation heterozygote and the products derived from alternate disjunction (orthoploid gametes) and from adjacent I disjunction (aneuploid I gametes). The numbers 2 and 3 stand for the centromere regions of the normal second and third chromosomes, respectively, 2′ and 3′ for the homologous regions of the translocated chromosomes, S denotes 2R, and L′ represents 3L of the translocated chromosome. It is implied, of course, that the unmarked arms, 2R and 3R, as well as 3L of the normal chromosome, are always associated with the appropriate centromere.

region (INT, fig. 1) as well as those in which an exchange has not occurred. From no-exchange tetrads (fig. 1), alternate disjunction of chromosomes will give rise to two orthoploid gametes, namely, $2S + 3$ and $2'L' + 3'$ while the aneuploid I gametes, $2S + 3'$ and $2'L' + 3$, arise following adjacent I disjunction. Other types of disjunction in this type of translocation are rare. The recovery of any one type of gamete from the female depends upon the fertilization of the egg by a sperm carrying the complementary type so that the zygote will have a balanced gene complement.

Following an exchange in the interstitial region (fig. 2) so-called asymmetric dyads are produced; that is, the chromatids making up the dyad associated with centromere 2 are of unequal length, S being shorter than L′, and, similarly, the dyad linked with centromere 2′ consists of a shorter and a longer chromatid. It is under just these conditions that nonrandomness of recovery of chromatids has been shown to hold for the X-chromosome, the shorter chromatid being recovered about twice as frequently as the longer. To understand the significance of this expected inequality as it bears upon the interpretation of the discrepancy between complementary aneuploid I classes, the constitution of the gametes following alternate and adjacent I disjunctions should be considered. The gametic products from alternate disjunction (fig. 3) are (1) the noncrossover orthoploids, O_1 and O_2 and (2) the crossover aneu-

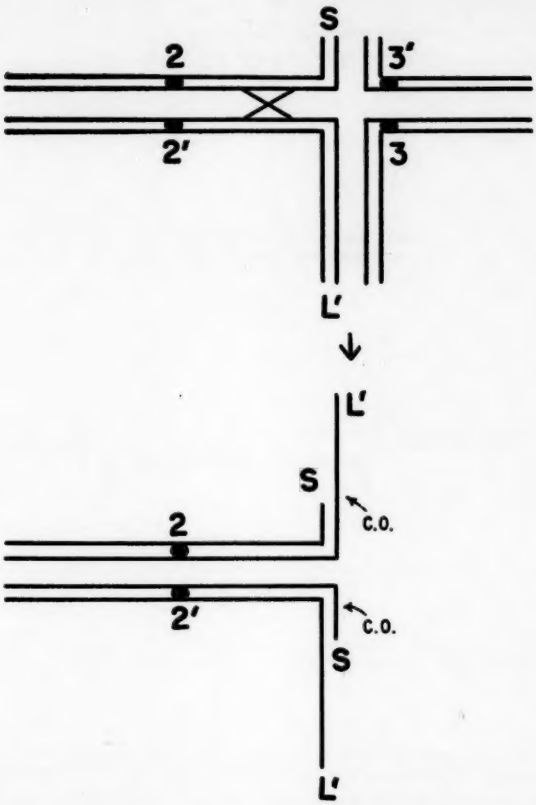

FIGURE 2.—The constitution of the crossover dyads following an exchange in the interstitial region. Note the dissimilarity in the length of the chromatids making up each of these dyads. The symbol *c.o.* stands for a crossover chromatid.

ploids, A_1 and A_2. Type A_1, carrying $2L'$, is expected to be produced less frequently than O_1 which contains 2S, and A_2, in which is included $2'S$, should be formed in excess of the type bearing $2'L'$, i.e. O_2. Consequently, of the complementary crossover aneuploids, the type carrying $2L' + 3$ will be recovered in the offspring less frequently than that containing $2'S + 3'$. Although these gametes, when recovered, are recognizable as crossovers when the interstitial region is genetically marked, they would be included in the general class of noncrossovers in situations where crossing over in this region is not followed, that is, they would be recovered in the classes receiving $2'L' + 3$ and $2S + 3'$, respectively, from the female. As was indicated above, these were the discrepant classes found in GLASS' experiments.

From adjacent I disjunction (fig. 4), the noncrossover aneuploids, A_3 and A_4, and

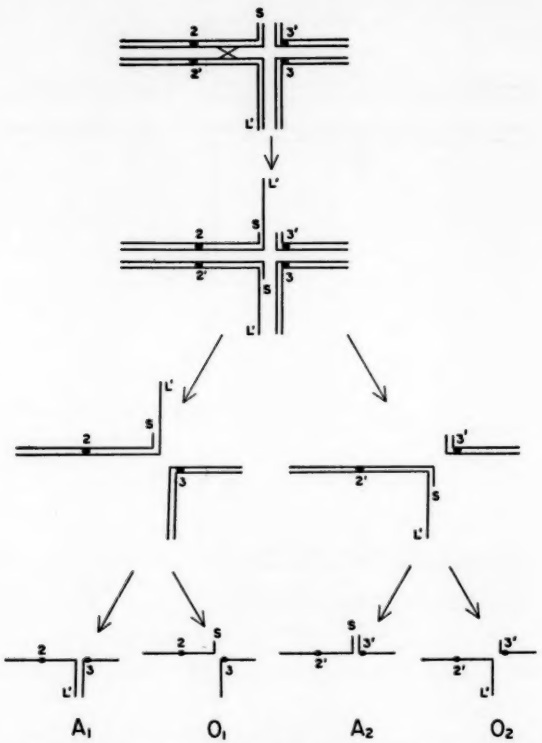

FIGURE 3.—The events leading to the production of complementary aneuploid I gametes (A_1 and A_2) following alternate disjunction at the first division, where A_2, carrying the shorter crossover chromatid is expected to be produced more frequently than A_1 which bears the longer.

the crossover orthoploids, O_3 and O_4, are produced. Assuming the same relationships to hold under these circumstances as those postulated as existing between gametes following alternate disjunction, then the complementary noncrossover aneuploids from exchange tetrads should be recovered with different frequencies. Hence, an inequality in the relative frequencies of complementary noncrossover aneuploids could be inferred to be a consequence of a differential recovery of these types from exchange tetrads. It should be noted that a discrepancy similar to that expected in the complementary crossover aneuploids would be found in the complementary crossover orthoploids since both types come from situations in which an asymmetric dyad is formed. The magnitude of the inequality between complementary noncrossover orthoploids will depend on the rate of alternate disjunction from no-exchange vs. that from exchange tetrads, that is, the greater the former, the smaller the discrepancy and *vice versa*. In brief, then, the following points may be emphasized: (1) the discrepancy between complementary aneuploid I types is interpretable on the basis of non-

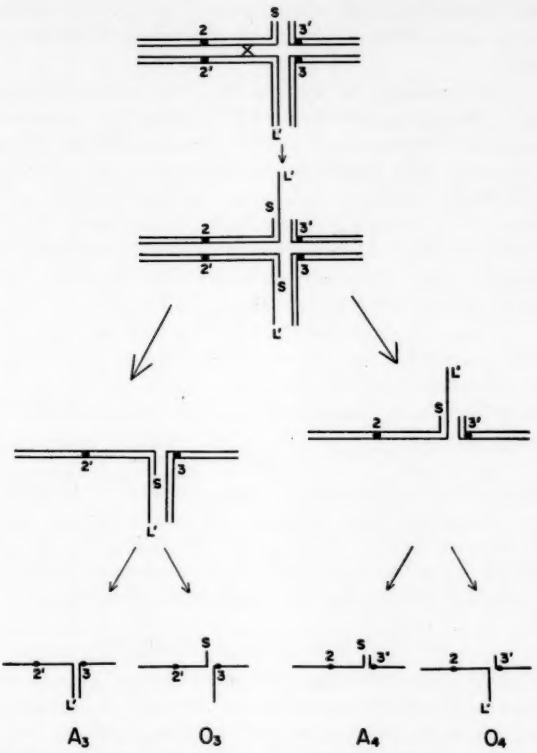

FIGURE 4.—The consequences of adjacent I disjunction following an exchange in the interstitial region.

random disjunction of the structurally dissimilar chromatids making up the asymmetric dyad, such that the shorter is included more frequently than the longer in the egg nucleus; (2) a difference in the frequency of recovery is expected between those complementary aneuploid types coming from exchange tetrads (no difference being expected from those which arise from no-exchange tetrads) and (3) crossover aneuploid I gametes are recovered following alternate disjunction while crossover orthoploid gametes are produced when adjacent I disjunction follows an exchange that is, a reversal of the relationship between the type of disjunction and the kinds of resultant gametes found in the absence of an exchange.

The regular conversion of orthoploid gametes into aneuploid gametes (and vice versa) by the occurrence of an exchange in the interstitial region makes it impossible to relate the recovered gametic frequencies to frequencies of the disjunctional types, alternate vs. adjacent, unless the interstitial region is so marked that the exchange frequency may be estimated. NOVITSKI (1951) pointed out that in a translocation of

the type under consideration here, with an extensive interstitial region, disjunction could be entirely of the alternate type, with the aneuploid gametes originating in exchange tetrads.

The results from experiments set up to throw some light on the various questions raised in the preceding paragraphs showed the following: (1) a non-random distribution of chromosomes occurs such that following an exchange in the interstitial region, the deficient crossover class, made up of individuals bearing the longer crossover chromatid, appears only about one half to one third as frequently as does the complementary class; (2) a discrepancy is found only between complementary crossover types while complementary noncrossovers are recovered with equal frequency; (3) the rate of crossing over in the interstitial region was surprisingly low, (about 12–15%) and possibly related to this was the absence in the data of an inequality of the magnitude of that found in earlier reports and (4) a high frequency of crossover individuals identified as coming from adjacent I disjunction following an exchange in the interstitial region are recovered.

A more detailed account of these experiments is presented below.

DESCRIPTION OF THE TRANSLOCATION

The translocation used in the present study, *T(2;3)bw*^{V4}, abbreviated as *V4*, is associated with a dominant brown variegated eye color, and involves an interchange of unequal segments, the region from the locus of *bw* (2-104.5) to the tip of 2R having been exchanged for approximately all of 3L. Individuals heterozygous for the translocation are viable and fertile while the translocation homozygotes are semilethal.

RESULTS FROM GLASS' EXPERIMENTS

GLASS (1934, 1935) found that when *V4 st/st* females were crossed by *V4 st Pr/Cy C(3)x* males a striking inequality was observed between the numbers of recovered individuals in the complementary aneuploid I classes, namely, 1242 *V4* and 709 *Cy V4 st Pr* (table 1a). That differential viability was not responsible for this unexpected finding was shown in the results from the reciprocal cross (table 1b) where it is obvious that the difference disappears, the two types being recovered with equal frequency, 608 *V4* and 609 *Cy V4 st Pr*. GLASS proposed the following explanation to account for the difference in the results from the reciprocal crosses. Since the *V4* translocation had been kept in a balanced condition, newly arisen lethals could accumulate in the translocation stocks. If a recessive lethal were located in L′ (3L), crossing over could shift the lethal to the homologous arm so that a gamete of the constitution 2′L′ + 3 might have a lethal in each of the 3L limbs; individuals homozygous for the lethal would, of course, be eliminated. Thus, according to this idea, the reason for the different results in the two experiments lies in the presence of an inversion, *C(3)x*, in the female inhibiting crossing over in L′ in one case, and its absence in the female in the other.

It was pointed out by GLASS that this explanation was not entirely satisfactory because the expected loss from homozygosis (theoretical maximum, 25%) would not be sufficient to account for the magnitude of the observed deficiency (about 43%). It might be pointed out further that since the lethal postulated as being present in L′

TABLE 1

Progeny of V4 st/st *females by* V4 st Pr/Cy C(3)X *males* (a) *and of the reciprocal cross* (b) (*aneuploid II types are omitted*)

Composition of gametes		Progeny phenotype	Number	
Male	Female		(a)	(b)
orthoploid				
2′L′ + 3′	2S + 3	*V4 st Pr*	1789	963
2S + 3	2S + 3	*Cy*	1900	887
2S + 3	2′L′ + 3′	*Cy V4*	1700	936
2′L′ + 3′	2′L′ + 3′	*V4/V4 st Pr*	273	240
aneuploid I				
2′L′ + 3	2S + 3′	*V4*	1242	608
2S + 3′	2′L′ + 3	*Cy V4 st Pr*	709	609

in the female would presumably be present also in L′ in the male, an exchange, shifting the position of the lethal to the homologous arm would eliminate those orthoploid individuals receiving 2S + 3(*1*) from the female and 2 L + 3 (*1*) from the male. Consequently, a marked deficiency would be expected in the *V4 st Pr* class; inspection of table 1a will show that this is not the case.

DEMONSTRATION OF NONRANDOM DISJUNCTION

Females of the constitution *V4 st/ stw*^{3} *c st* were crossed by normal males homozygous for *stw*^{3} *c st*. If adjacent I disjunction takes place after an exchange in the interstitial region, the gametes carrying the crossover products, 2L′ + 3′ and 2′S + 3 are recoverable since fertilization by a sperm carrying 2S + 3 gives rise to a zygote having a normal complement of genes. As a consequence of nonrandom disjunction, the gametes carrying 2′S should be recovered more often than those carrying 2L′. On the other hand, if adjacent I disjunction does not occur, the crossover products are not recoverable since they become aneuploid gametes and under the conditions of the experiment would give rise to inviable zygotes. The cross is diagrammed in figure 5 and the results are given in table 2. It is seen that crossover individuals are recovered. Of 2323 crossovers in Region I, *stw*^{3}—*c*, 1530 involved the shorter chromatid and 793 the longer, and for Region II, *c*—breakpoint, 529 involved the shorter and 278 the longer chromatid.

The results from an experiment to determine the extent to which differential viability might have been responsible for this discrepancy between complementary classes are given in table 3 (fig. 5). It may be noted on comparing the two experiments that whereas in the former, the classes carrying the longer crossover chromatid also carried a progressively greater number of mutants, in the latter, the reverse is the case. If a change in the distribution of mutants had the effect of appreciably affecting viability, one might expect to find a striking difference in the degree of non-randomness between complementary crossovers for Region I as compared with those from Region II; it is obvious upon inspection that this is not the case. Attention might

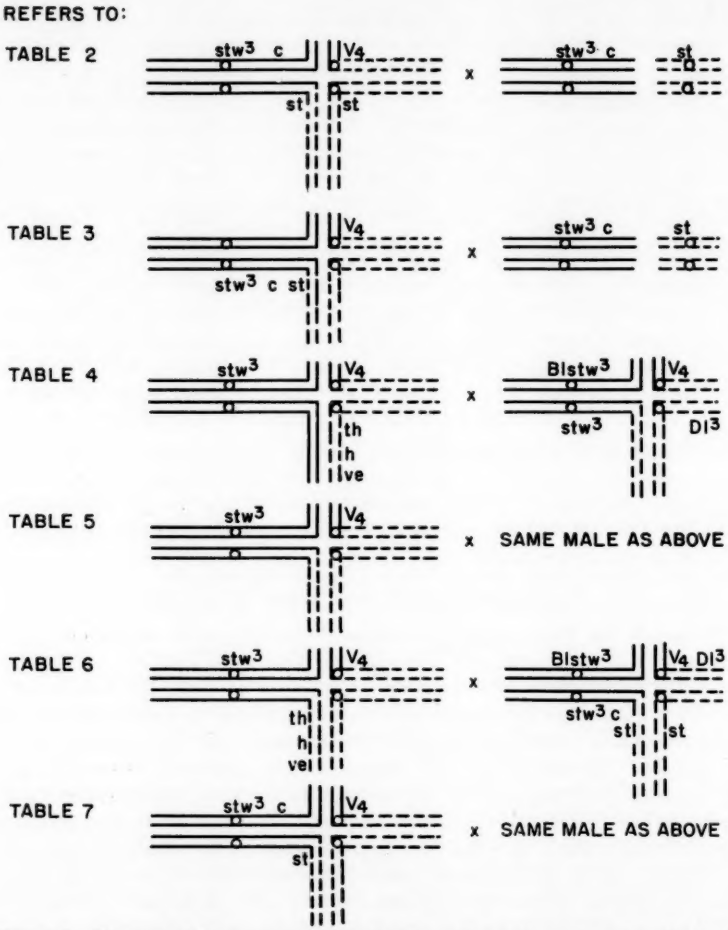

FIGURE 5.—Diagrams of the crosses made showing the position of the markers. In each case the female parent is on the left. In translocation heterozygotes the normal second chromosome is the upper left, the normal third is lower right, and the other two are translocation chromosomes.

be called to the classes *st* and *V4 stw³ c* in table 3. These represent complementary crossover products of an exchange between *st* and the breakpoint, that is, not in the interstitial region but in arm L′, their recovered numbers being 105 and 95, respectively. This kind of result is not inconsistent with the non-random interpretation since an exchange not within the limits of the interstitial region occurs between structurally identical chromatids.

TABLE 2

Progeny of V4 st/ stw^{3}c st *females by* stw^3 c st *males. In each case, the composition of the male gamete is 2S + 3*

Composition of female gamete	Progeny phenotype	Number
	non-c.o.	
2S + 3	*stw^3 c st*	8691
2′L′ + 3′	*V4 st*	7616
	c.o. Reg. I	
2′S + 3	*c st*	1530
2L′ + 3′	*V4 stw^3 st*	793
	c.o. Reg. II	
2′S + 3	*st*	529
2L′ + 3′	*V4 stw^3 c st*	278
	c.o. Regs. I, II	
2S + 3	*stw^3 st*	17
2′L′ + 3′	*V4 c st*	29

RESULTS FROM CROSSES OF TRANSLOCATION FEMALES BY TRANSLOCATION MALES

The discussion to follow will be concerned with an analysis of results from experiments designed to test the following possibilities: (1) that there is a striking discrepancy in the relative frequencies of the complementary aneuploid I classes and that this may be accounted for on the basis of homozygosis for a lethal in L' or (2) that there is no discrepancy of this order between aneuploid I individuals which do not arise from an exchange in the interstitial region, but that one does exist between the classes representing detectable crossover products arising from an exchange in this region, the discrepancy resulting from the operation of the non-random effect.

The presence or absence of a lethal located at the tip of L' at approximately the same position postulated by GLASS, may be inferred by comparing the results from experiments in which this arm is retained with those in which it is replaced with a normal arm. In the former situation, an appreciable deficiency should appear in the aneuploid I class of the composition 2′L′ + 3/2S + 3′ (female gamete/male gamete) as compared with the complementary class, 2S + 3′/2′L′ + 3. Contrarily, this discrepancy should disappear as a result of the removal of this chromosome and its replacement with a normal equivalent. For information on the contribution which might be made to the discrepancy from the loss of individuals as a result of homozygosis for this lethal, this arm may be marked genetically and the frequency of homozygosis determined for some distally located mutant; in two experiments, a chromosome carrying *ve h th* was used for this purpose. The results from the experiments in which L' was retained are given in tables 4 and 5 (fig. 5). In these cases,

TABLE 3

Progeny of V4 stw^3 c st/+ *females by* stw^3 c st *males. In each case, the composition of the male gamete is 2S + 3*

Composition of female gamete	Progeny phenotype	Number
	non-c.o.	
2S + 3	+	2201
2′L′ + 3′	*V4 stw^3 c st*	1922
	c.o. Reg. I	
2′S + 3	*stw^3*	424
2L′ + 3′	*V4 c st*	170
	c.o. Reg. II	
2′S + 3	*stw^3 c*	147
2L′ + 3′	*V4 st*	68
	c.o. Regs. I, II	
2S + 3	*c*	16
2′L′ + 3′	*V4 stw^3 st*	10
	c.o. st-bp	
2S + 3	*st*	105
2′L′ + 3′	*V4 stw^3 c*	95
	c.o. Reg. I, st-bp	
2′S + 3	*stw^3 st*	21
2L′ + 3′	*V4 c*	5

Cy/V4 males were mated to *stw^3*; *ve h th* females in one instance, and to *stw^3* females in the other, and the non-*Cy* female offspring crossed by appropriate males. When *L′* was replaced with an arm carrying *ve h th*, and by one carrying *st*, the results that were obtained are those given in tables 6 and 7 (fig. 5).

Since neither *L′* nor the interstitial region was marked genetically in the tests made by GLASS, crossovers in these regions could not be recognized as such. These individuals appeared as noncrossovers and therefore were classified as belonging to some general class which included noncrossovers as well as undetected crossovers. To illustrate the kind of result which would have been expected under these conditions, the noncrossover and corresponding crossover classes from the present experiments have been added together. These totals are given in tables 4–7 in the column headed "Regrouped Classes". Thus, for example, as shown in table 5, each of the complementary aneuploid I classes, is a mixture of individuals identified as noncrossovers and those identified as crossovers. In one are included 1110 noncrossovers, *V4 stw^3 Dl^3*, and 331 *V4 Dl^3* individuals carrying the shorter crossover chromatid, while the other includes 824 noncrossovers, *Bl V4*, and 125 *Bl V4 stw^3*

TABLE 4

Progeny of V4/stw^3 ve h th *females by* V4 stw^3/Bl stw^3 Dl^3 *males*

Composition of gametes		Progeny phenotype	Number	Regrouped classes	Correction
Male	Female				
non-c.o. orthoploid					
2S + 3	2S + 3	*Bl Dl^3 stw^3*	299	339	274
2′L′ + 3′	2S + 3	*V4 stw^3*	331	355	306
2S + 3	2′L′ + 3′	*Bl V4 Dl^3*	282	302	271
2′L′ + 3′	2′L′ + 3′	*V4/V4*	54		
non-c.o. aneuploid I					
2′L′ + 3	2S + 3′	*V4 stw^3 Dl^3*	174	199	142
2S + 3′	2′L′ + 3	*Bl V4*	99	147	116
c.o. orthoploid					
2S + 3	2′S + 3	*Bl Dl^3*	40		
2′L′ + 3′	2′S + 3	*V4*	24		
2S + 3	2L′ + 3′	*Bl V4 Dl^3 stw^3*	20		
c.o. aneuploid I					
2′L′ + 3	2′S + 3′	*V4 Dl^3*	25		
2S + 3′	2L′ + 3	*Bl V4 stw^3*	11		
c.o. in L′					
2S + 3′	2′L′ + 3	*Bl V4 ve..*	32		
c.o. in interstitial region and in L′					
2S + 3′	2L′ + 3	*Bl V4 stw^3 ve..*	5		

individuals carrying the longer crossover chromatid. The ratio of these classes to one another from experiments in which L' was not replaced are 199/147 and 1441/949, differences of 26.1% and 34.1%, respectively. From those experiments in which L' was replaced with a normal equivalent, they are 782/527 and 663/497, differences of 32.5% and 25.0%, respectively. The information on the frequency of homozygosis for *ve* is given in table 4, 37/147 or 25.2%, and in table 6, 104/527 or 19.6%.

These two results, the failure to modify the discrepancy by replacing the section of the chromosome, presumed to carry the lethal, by a homologous section of a chromosome from another stock, and the relatively low rate of homozygosis for the distal marker *ve*, provide experimental evidence that homozygosity for lethals is not the cause of the discrepancy.

INTERPRETATION OF THE RESULTS ON THE BASIS OF THE NON-RANDOM HYPOTHESIS

Although it is not possible to determine directly the relative frequencies with which noncrossover individuals come from exchange and no-exchange tetrads, these

TABLE 5

Progeny of V4/stw^3 *females by* V4 stw^3/ Bl stw^3 Dl^3 *males*

Composition of gametes		Progeny phenotype	Number	Regrouped classes	Correction
Male	Female				
		non-c.o. orthoploid			
2S + 3	2S + 3	*Bl Dl^3 stw^3*	2046	2512	1715
2′L′ + 3′	2S + 3	*V4 stw^3*	2202	2608	1871
2S + 3	2′L′ + 3′	*Bl V4 Dl^3*	1966	2164	1841
2′L′ + 3′	2′L′ + 3′	*V4/V4*	189		
		non-c.o. aneuploid I			
2′L′ + 3	2S + 3′	*V4 stw^3 Dl^3*	1110	1441	674
2S + 3′	2′L′ + 3	*Bl V4*	824	949	626
		c.o. orthoploid			
2S + 3	2′S + 3	*Bl Dl^3*	466		
2′L′ + 3′	2′S + 3	*V4*	406		
2S + 3	2L′ + 3′	*Bl V4 Dl^3 stw^3*	198		
		c.o. aneuploid I			
2′L′ + 3	2′S + 3′	*V4 Dl^3*	331		
2S + 3′	2L′ + 3	*Bl V4 stw^3*	125		

may be inferred from those of the recovered crossover types. For example, after adjacent disjunction following an exchange in the interstitial region, the gametes 2L′ + 3′ and 2S + 3′ are formed from the dyads 2S:2L′ + 3′ (the chromatids of the dyad are separated by a colon) and gametes of the constitution 2′S + 3 and 2′L′ + 3 are produced from the complementary dyads, 2′S:2′L′ + 3. Now, from the frequencies with which the crossover types 2L′ + 3′ and 2′S + 3 are recovered may be inferred those of the noncrossover types, 2S + 3′ and 2′L′ + 3, respectively, which have come from exchange tetrads. Subtracting this number from the total number of noncrossover individuals, gives a figure representing the number of noncrossovers which came from no-exchange tetrads. A consideration, from this aspect, of the data for the complementary aneuploid classes from table 5 will serve to illustrate the nature of this correction. From the kind of situation just described, that is, from adjacent disjunction at the first divisiom, 198 2L′ + 3′, *Bl V4 Dl^3 stw^3*, individuals representing the recovery of the longer crossover chromatid, and an average of 436 2′S + 3, 466 *Bl Dl^3* and 406 *V4*, individuals carrying the shorter crossover chromatid appeared. Hence, 436 offspring carrying the shorter noncrossover chromatid, 2S + 3′, *V4 stw^3 Dl^3*, and 198 carrying the longer, 2′L′ + 3, *Bl V4*, are calculated to have come from exchange tetrads. Thus, 674 *V4 stw^3 Dl^3* (1110-436) and 626 *Bl V4* (824-198) represent the number of noncrossovers which came from no-exchange tetrads. The results of applying this method of correction for orthoploid and aneuploid noncrossover classes are given in table 4–7 in the column headed "Correction". The ratios of

TABLE 6

Progeny of V4 ve h th / stw^3 *females by* V4 Df^3 stw^3 c st / Bl stw^3 c st *males*

Composition of gametes		Progeny phenotype	Number	Regrouped classes	Correction
Male	Female				
non-c.o. orthoploid					
2S + 3	2S + 3	*Bl stw^3*	913	1116	757
2′L′ + 3′	2S + 3	*V4 stw^3 Df^3*	908	1102	752
2S + 3	2′L′ + 3′	*Bl V4*	942	1027	886
2′L′ + 3′	2′L′ + 3′	*V4/V4*	40		
non-c.o. aneuploid I					
2′L′ + 3	2S + 3′	*V4 stw^3 st*	626	782	428
2S + 3′	2′L′ + 3	*Bl V4 Df^3*	378	527	397
c.o. orthoploid					
2S + 3	2′S + 3	*Bl*	203		
2′L′ + 3′	2′S + 3	*V4 Df^3*	194		
2S + 3	2L′ + 3′	*Bl V4 stw^3*	85		
c.o. aneuploid I					
2′L′ + 3	2′S + 3′	*V4 st*	156		
2S + 3′	2L′ + 3	*Bl V4 Df^3 stw^3*	45		
c.o. in L′					
2S + 3′	2′L′ + 3	*Bl V4 Df^3 ve..*	93		
c.o. in interstitial region and in L′					
2S + 3′	2L′ + 3	*Bl V4 Df^3 stw^3 ve..*	11		

individuals in the complementary aneuploid I classes which came from no-exchange tetrads are 142/116 (table 4), 674/626 (table 5), 428/397 (table 6), and 442/399 (table 7). It may be seen that the effect of the correction on the relative frequencies of complementary noncrossover orthoploids for those coming from exchange tetrads is less striking since the number of crossovers relative to that of the noncrossovers is considerably less than that for the aneuploid classes. However, the data given in tables 6 and 7 that there is a statistically significant difference between the classes *V4 stw^3 Df^3* and *Bl stw^3* on the one hand, and *Bl V4* on the other, of some importance being the approximate 16% excess of *Bl V4* as compared with *V4 stw^3 Df^3*. Since this probably reflects a difference in the relative viabilities of the two classes, it would mean that the frequency of individuals from the class *V4 stw^3 Df^3* in tables 4 and 5 which came from no-exchange tetrads should be increased by 16%. It may be shown, however, that this correction is not sufficient to effect a difference between these complementary aneuploid classes and those from tables 6 and 7. (A comparison

TABLE 7

Progeny of V4 st / stw^3 c *females by* V4 Dl^3 stw^3 c st / Bl stw^3 c st *males*

Composition of gametes		Progeny phenotype	Number	Regrouped classes	Correction
Male	Female				
non-c.o. orthoploid					
2S + 3	2S + 3	*Bl stw^3 c*	857	957	747
2′L′ + 3′	2S + 3	*V4 Dl^3 stw^3 c*	832	953	722
2S + 3	2′L′ + 3′	*Bl V4 st*	882	943	845
2′L′ + 3′	2′L′ + 3′	*V4/V4*	7		
non-c.o. aneuploid I					
2′L′ + 3	2S + 3′	*V4 stw^3 c st*	553	663	442
2S + 3′	2′L′ + 3	*Bl V4 Dl^3*	460	497	399
c.o. orthoploid Reg. I					
2S + 3	2′S + 3	*Bl c*	84		
2′L′ + 3′	2′S + 3	*V4 Dl^3 c*	101		
2S + 3	2L′ + 3′	*Bl V4 stw^3 st*	54		
c.o. orthoploid Reg. II					
2S + 3	2′S + 3	*Bl*	16		
2′L′ + 3′	2′S + 3	*V4 Dl^3*	20		
2S + 3	2L′ + 3′	*Bl V4 stw^3 c st*	7		
c.o. aneuploid Reg. I					
2′L′ + 3	2′S + 3′	*V4 c st*	91		
2S + 3′	2L′ + 3	*Bl V4 Dl^3 stw^3*	29		
c.o. aneuploid Reg. II					
2′L′ + 3	2′S + 3′	*V4 st*	19		
2S + 3′	2L′ + 3	*Bl V4 Dl^3 stw^3 c*	8		

of the results in table 4 with those in tables 6 and 7 give χ^2 values of 3.87 and 3.17, 2 d.f., P values of 0.2–0.1 and 0.3–0.2, respectively; a comparison of results in table 5 with those in tables 6 and 7 gives χ^2 values of 28.2 and 1.82, 2 d.f., P values of 0.3–0.2 and 0.5–0.3, respectively).

FREQUENCIES OF THE VARIOUS RECOVERED CLASSES

In comparing our results with those obtained by GLASS insofar as the relative frequencies of orthoploids and aneuploids are concerned, the gametic frequencies of the two classes were calculated from the present data by taking the square root of the average number of individuals in each class, that is, by assuming that the frequency of the two types of gametes are equal in the male and the female, and applying the appropriate correction for the viability difference between the complementary

TABLE 8

Summary of crossover types and frequencies from the tables indicated. The classes bearing identical symbols in each table have been combined

Symbol	Disjunction	Chromatid	Tables			
			4	5	6	7
2′L′ + 3 / 2′S + 3′	exchange alternate	short	25	331	156	110
2S + 3′ / 2L′ + 3	exchange alternate	long	11	125	56	37
2S + 3 / 2′S + 3	exchange adjacent	short	40	466	203	110
2′L′ + 3′ / 2′S + 3	exchange adjacent	short	24	406	194	121
2S + 3 / 2L′ + 3′	exchange adjacent	long	20	198	85	61

aneuploid classes in tables 4 and 5. These ratios from the columns headed "Regrouped classes" are as follows: 1.0:0.75 (table 4), 1.0:0.72 (table 5), 1.0:0.77 (table 6), and 1.0:0.78 (table 7). Calculated in the same way, the ratio of orthoploids to aneuploids from the results obtained by GLASS from crosses involving heterozygous *V4* females in the absence of inversions (see table 1a), is 1.0:0.74. As has been shown, however, these classes are composed of a mixture of noncrossover and crossover types, each of these types coming from a different kind of disjunction; that is, crossover individuals identified as orthoploids come from adjacent I disjunction while crossover aneuploids come from alternate disjunction. Therefore, the ratio of the recovered (i.e., not gametic) frequencies of orthoploids to aneuploids from no-exchange tetrads and from exchange tetrads was calculated. From no-exchange tetrads, the ratios of orthoploids to aneuploids are: 1.0:0.49 (table 4); 1.0:0.39 (table 5); 1.0:0.52 (table 6); and 1.0:0.55 (table 7). It is clear that orthoploids coming from no-exchange tetrads following alternate disjunction appear considerably more frequently than do aneuploids from no-exchange tetrads following adjacent I disjunction. Since following an exchange, crossover aneuploids come from alternate disjunction, and crossover orthoploids from adjacent disjunction the terms "exchange alternate" and "exchange adjacent" have been used to designate the types of disjunction giving rise to aneuploid and orthoploid crossovers, respectively. From the summary given in table 8 of the results from tables 4–7, the ratios, in that order, of the total crossover orthoploids (exchange adjacent) to crossover aneuploids (exchange alternate) are: 1.0:0.64, 1.0:0.64, 1.0:0.66, and 1.0:0.76. These ratios indicate that, after an exchange in the interstitial region, the crossover classes identified as having come from adjacent I disjunction are recovered with a greater frequency than those which came from alternate disjunction. The ratios of complementary crossover products for each of the crossover types are as follows: (1) for crossover orthoploids, the ratios of individuals carrying the shorter crossover chromatid to those carrying the longer are 1.0:0.63, 1.0:0.45, 1.0:0.43, and 1.0:0.55 (2) for crossover aneuploids, the comparable ratios are 1.0:0.44, 1.0:0.38, 1.0:0.36, and 1.0:0.34.

CONCLUSIONS

The results from these experiments show that the discrepancy between complementary aneuploid I classes is not related to the elimination of individuals carrying a lethal in the homozygous condition, since, as indicated by comparing the results from two types of experiments designed specifically to test this, there is no evidence for the presence of a lethal in the translocation stocks used. It is evident, however, that a discrepancy as large as that reported by GLASS was not found. The hypothesis of nonrandom disjunction is supported by these results since this phenomenon was demonstrated as operating in exchange tetrads. It was shown that adjacent I disjunction may take place following an exchange in the interstitial region, and that the great majority of aneuploid individuals come from no-exchange tetrads. Disagreements between the data presented here and those presented previously by GLASS (1933) are at least partially resolvable if the following argument is made, based on the assumption of different crossover rates in the two experiments. The extent to which nonrandom disjunction may contribute to the discrepancy between complementary aneuploid I classes will depend not only on the degree of nonrandomness but also on the frequency of crossing over in the interstitial region. A higher crossover value would have the effect of increasing the difference between complementary aneuploid classes and reducing the frequency of noncrossover aneuploids coming from no-exchange tetrads. As the simplest approximation, a direct relationship of the following sort may be set up to arrive at a value for the discrepancy to be expected between the complementary aneuploid classes after regrouping assuming a higher frequency of crossing over: the observed difference between complementary aneuploid noncrossovers/the difference after regrouping = the expected difference between complementary aneuploid noncrossovers resulting from a higher crossover rate/x, the value for the expected difference after regrouping. The crossover value used was 21.9%, given by GLASS (1933). The results of these calculations, after applying the viability correction in tables 5 and 6, give the following values for x: 53.0% (table 4), 49.4% (table 5), 42.5% (table 6), and 37.8% (table 7); the average difference observed by GLASS was 42.9%. The general agreement of these calculated values suggests that the apparent differences in the frequency of adjacent disjunction, and the extent of non-randomness may be the result of some change, with the effect of simply altering crossover values, taking place in the lines used between the time of the early experiments (1933), and those reported on here.

It was pointed out that whereas no-exchange orthoploids coming from alternate disjunction appeared appreciably more frequently than the no-exchange aneuploids coming from adjacent disjunction, the orthoploid crossovers, identified as coming from adjacent I disjunction appeared more frequently than aneuploid crossovers identified as having come from alternate disjunction. An explanation which can be proposed to explain these results involves a shift in the relative positions of centromeres 2 and 2′ after an exchange in the interstitial region such that each would occupy the position of the former. This would have the effect of placing in an alternate position the dyads which were originally arranged in an adjacent fashion and *vice versa*. If alternately disposed chromosomes disjoin more frequently to the same pole than those arranged adjacently, then the gametes arising from this kind of

disjunction (and which carry orthoploid crossover products) would be recovered more often than those coming from adjacent disjunction (containing aneuploid crossover products). The results might equally well be interpreted, however, assuming a shift prior to the time of crossing over. The data do not permit distinguishing between these possibilities.

SUMMARY

Female Drosophila heterozygous for certain kinds of translocations do not produce complementary types of gametes with the expected equal frequencies. A genetic analysis has shown that the inequality of frequencies arises after an exchange occurs in the interstitial region, which forms asymmetric dyads consisting of a short and a long chromatid. Non-random disjunction is responsible for the recovery of the shorter chromatid in preference to the longer, thereby causing a deviation from the simple one-to-one expectations for the frequency of complementary types. Other aspects of disjunction in this particular kind of translocation heterozygote are discussed.

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RADIATION-INDUCED GENETIC SEGREGATIONS IN VEGETATIVE CELLS OF DIPLOID YEAST

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ULTRAVIOLET irradiation frequently induces homozygosis at one or more loci in heterozygous vegetative cells of *Saccharomyces cerevisiae*. For a particular allelic pair, the observed frequency of such inductions can approach two percent among survivors under conditions where fewer than fifty percent of the irradiated cells are killed. These alterations are almost certainly due to genetic segregation rather than to gene mutation, since: (1) complementary types (homozygous recessive and homozygous dominant) often appear as different lines of descent from a single irradiated heterozygote, (2) irradiated haploids show only rare changes from dominant to recessive, and (3) irradiated homozygous recessive cells show only rare changes to heterozygous (JAMES 1953; 1955). However, the observed homozygosis could result either from independent segregations of individual loci or from linked segregations involving whole chromosomes or parts of chromosomes. The present investigation was undertaken to obtain information on the mechanism by which the segregants are produced.

MATERIAL AND METHODS

Allelic gene pairs at five loci were used in this study. Of these, two were concerned with galactose utilization; one recessive allele (g_1) has been described previously (JAMES 1955), the other (g_2) was obtained by ultraviolet irradiation of a haploid strain. Absence of the dominant allele at either locus results in the recessive phenotype. Genes at the other three loci were associated with sex (*A*, *a*), and with non-requirement vs. requirement for uracil (*Ur*, *ur*) and for tryptophan (*Tr*, *tr*). The latter were obtained through the courtesy of DR. S. SPIEGELMAN.

The genes affecting galactose utilization were used for the initial detection of induced homozygotes. The isolation of variant cells homozygous recessive for galactose was easily accomplished, because dominant and recessive phenotypes differ in colony morphology on a galactose-EMB indicator medium; homozygous dominant or heterozygous cells form black (positive) colonies, whereas homozygous recessive cells form white (negative) colonies. The variant colonies that appear when heterozygous cells are irradiated and plated on this medium are either wholly white or sectored for black and white.

Isolates for genetic analysis were obtained by picking, dilution, and replating of individual colonies, followed by single colony isolation. Segregation analyses were confined to those isolates that did not contain radiation-induced recessive lethals. The segregants (either 3 or 4) from a single four-spored ascus were used to determine genotype if these indicated heterozygosity at the loci under study. This number was considered adequate in view of the consistent results of previous individual analyses

(JAMES 1955). However, in the case of those isolates where radiation-induced homozygosis was indicated, all four segregants from each of a minimum of two asci were characterized in order to eliminate possible erroneous classifications resulting from occasional irregular segregations.

Tryptophan and uracil requirements were determined by plating several hundred cells in the presence and absence of these compounds; recessive genotypes were indicated by the complete absence of visible colonies on deficient media. Galactose and sex reactions were determined in the manner already described (JAMES 1955). Tests for induced homozygosity for sex were performed in the following manner. Ability of isolates to sporulate was determined. Inability to sporulate was considered an initial indication of homozygosity for sex. Such isolates were tested for mating reaction, and those that showed a positive reaction were considered to be diploids homozygous for sex. Diploidy of these isolates, rather than haploidy, was inferred from large cell size and from the fact that, with each of four such variants isolated previously, the zygotic cultures produced by matings with haploids sporulated abundantly but exhibited a low frequency of spore germination. The frequencies as estimated by the above procedure are suggestive only, since they are subject to errors of unknown magnitude.

Media and methods of irradiation were similar to those used previously (JAMES 1954). All crosses were made by the technique of mating single cells and isolating the resultant zygote (CHEN 1950).

RESULTS AND DISCUSSION

Information on the mechanism by which radiation-induced genetic segregations occur in vegetative cells can be obtained by comparison of the behaviour of linked and unlinked marker genes.

Using tetrad analysis, a survey of linkage relationships indicated that the five loci *A*, G_1, G_2, *Ur*, and *Tr*, together with centromeres, comprise three linkage groups and one unlinked locus (table 1). These are: (1) *A*—centromere, (2) G_1—centromere, (3) (G_2—*Tr*)—centromere, and (4) *Ur* independent. With the evidence that the locus of *Tr* is on the same chromosome as that of G_2 (no crossover segregants in 91 segregations) but on a different chromosome from that of G_1, it is possible to test whether the radiation-induced segregations involve (1) each locus independently of the others, or (2) linked (chromosomal) segregations.

Results of such a test (summarized in table 2) indicated that the segregations are chromosomal in nature; G_2 and *Tr* show linkage in induced somatic segregations whereas G_1 and *Tr* do not. Among g_2g_2 variant isolates (meiotic linkage with tryptophan) seven of eight were homozygous for tryptophan (*TrTr*), whereas among g_1g_1 variants (no meiotic linkage with tryptophan) none of 16 were homozygous for tryptophan. The data also suggest that groups of linked genes do not segregate simply as units (whole chromosome segregation) since the isolate from one negative (g_2g_2) variant colony of the eight studied was not homozygous for tryptophan.

Further evidence of the chromosomal nature of the somatic segregations was obtained from the positive portions of sectored colonies. The genotypes of positive isolates associated with six of the g_2g_2 variant colonies of table 2 were determined

TABLE 1

Linkage relationships between five loci in yeast as determined by tetrad analysis

Two-point segregations	Genotypes segregated*						Significant evidence of linkage‡
	Aa G_1g_1 G_2g_2 *Urur Trtr*			*Aa* G_1G_1 G_2g_2 *Urur Trtr*			
	Tetrad types†			Tetrad types			
	PD	NPD	T	PD	NPD	T	
G_1-*A*	10	13	19	—	—	—	G_1 to centromere; *A* to centromere
G_1-*Ur*	3	9	30	—	—	—	
G_1-*Tr*	15	18	9	—	—	—	G_1 to centromere; *Tr* to centromere
G_2-*A*	—	—	—	29	25	37	G_2 to centromere; *A* to centromere
G_2-*Ur*	—	—	—	18	12	61	
G_2-*Tr*	—	—	—	91	0	0	G_2 to *Tr*
A-*Ur*	6	9	27	13	14	64	
A-*Tr*	10	16	16	29	25	37	*A* to centromere; *Tr* to centromere
Ur-*Tr*	6	7	29	18	12	61	

* *Aa*—sex; G_1g_1, G_2g_2—galactose; *Urur*—uracil; *Trtr*—tryptophan.

† PD—parental ditype; NPD—non-parental ditype; T—tetratype. The genotype of the fourth segregant was inferred in 45 of the 133 segregations. Eleven irregular segregations were omitted from the data.

‡ Evidence of linkage to centromere is based on the hypothesis that a significant deviation of the ratio of non-parental ditype to tetratype segregations in excess of 1:4 indicates that the two loci are on different chromosomes and within 33 units of their respective centromeres (PERKINS 1953).

TABLE 2

Induced homozygosis in isolates from galactose negative variant colonies formed by irradiated cells of two genotypes, as determined by segregation analysis. Dose: 200 ergs/mm²

Pre-irradiation genotype: $\frac{A}{a}\frac{g_1}{G_1}\frac{G_2}{G_2}\frac{Tr}{tr}\frac{Ur}{ur}$		Pre-irradiation genotype: $\frac{a}{A}\frac{G_1}{G_1}\frac{g_2}{G_2}\frac{Tr}{tr}\frac{Ur}{ur}$	
No. colonies	Induced homozygosity	No. colonies	Induced homozygosity
13	g_1 only	5	g_2 and *Tr*
2	g_1 and *Ur*	2	g_2, *Tr* and *ur**
1	g_1 and *ur*	1	g_2 and *ur*

* The asci from one colony segregated 2:2 for growth vs. no growth on galactose. For this colony, genotype at uracil and tryptophan loci was determined on dextrose medium. The proposed genotype at the galactose locus was based on absence of galactose positive segregants in three complete segregations.

by tetrad analysis. Here again, linkage of the galactose and tryptophan loci was evident in the somatic segregants. In three of the six positive isolates the genotypes were entirely complementary to those of the negative isolates. In two of these, homozygosity for galactose and tryptophan (G_2G_2 *trtr*) in positive isolates matched the homozygosity (g_2g_2 *TrTr*) of the negative isolates. With the third colony, complementary homozygosity was extended to a third locus, that for uracil. In this instance the genotype of the positive isolate was *Aa* G_2G_2 *UrUr trtr*; that of the negative was *Aa* g_2g_2 *urur TrTr*. The positive clones from the other three colonies were heterozygous at all four loci.

As might be expected, phenotypically normal colonies from irradiated cells are unlikely to contain homozygous dominant cells. This has already been shown for G_1 (JAMES 1955), and was shown for G_2 in the present study. Here tryptophan requirement was used as an indication of homozygosity in galactose positive is lates; in G_2tr/g_2Tr diploids, any induced $trtr$ homozygotes would tend also to be G_2G_2 because of the close linkage between loci (table 1). Of the positive isolates from 24 sectored colonies, results indicated that 7 contained homozygous cells (mixed with heterozygous cells in 3 cases), whereas none of the isolates from 27 wholly positive colonies gave evidence of being homozygous. The assumed genotypes of the positive sectors were confirmed by tetrad analysis in six of the sectored colonies that were free of recessive lethals. (These six colonies provided the data on complementary homozygosis in the preceding paragraph.)

A hypothesis of genetic segregation requires an explanation of the presence of positive cells (in sectored colonies) whose genotype is non-complementary to that of the negative cells. In some instances their presence might be attributed to the plating of budding cells. However, the distribution of recessive lethals (JAMES 1955) precludes this as an overall explanation. Consequently, in at least some sectored colonies, the presence of noncomplementary positive cells must be attributed to a delay in genetic segregations until one or more mitotic divisions have occurred.

The absence of positive segregants (in wholly negative and many sectored colonies) also requires an explanation. There is evidence (JAMES 1954) that the occurrence of wholly negative colonies is due to the elimination of positive cells during early colony growth. Such eliminations would also account for the frequent absence of positive segregant cells in sectored colonies. Finally, it is of course likely that some fraction of radiation-induced variants results from mutation; in such cases one does not expect complementary genotypes to be present.

Any of several mechanisms can be involved in the production of chromosomal segregants. In artificially-synthesized diploid fungi spontaneous somatic segregations can result from mitotic crossing over and from haploid formation (PONTECORVO, GLOOR and FORBES 1954). Segregations in exceptional heterozygotes of *E. coli* apparently involve crossing over and haploid formation (LEDERBERG 1949). In Drosophila, both spontaneous (STERN 1936) and radiation-induced (WHITTINGHILL 1951) somatic segregations are attributable to mitotic crossing over. The possible importance of four mechanisms will be considered here. These are (1) reciprocal translocation between homologous chromosomes as a result of chromatid breakage, (2) induced meiosis with subsequent fusion to restore the diploid condition, (3) first meiotic division (disjunction of homologous centromeres), and (4) mitotic crossing over (disjunction of sister centromeres).

Chromatid breakage resulting in reciprocal translocations between homologous chromosomes could give rise to genetic segregations. However, any lack of precision in the exchange of chromosomal segments would produce a duplication in one segregant and a corresponding deficiency in the other The latter would be evident as a recessive lethal in one of the two lines of descent. Actually, no such condition has been found; of nine paired (i.e. complementary) segregants studied so far, recessive lethals have either been present in both lines of descent or in neither.

TABLE 3

*Recessive homozygosis for uracil and tryptophan, and homozygosis for sex, in isolates from induced galactose negative colonies**

Pre-irradiation genotype	Negative isolates from wholly negative or sectored colonies					Positive isolates from sectored colonies		
	No. of isolates	*ur*	*A*	*a*	*tr*	No. of isolates	*ur*	*tr*
a G_1 G_2 *tr ur*/*A* g_1 G_2 *Tr Ur*	197	9	3	4	3	67	3	2
a G_1 G_2 *Tr Ur*/*A* g_1 G_2 *tr ur*	281	11	17	6	6	121	8	3
	478	20	20	10	9	188	11	5

* Recessive homozygosity at loci for uracil and tryptophan was determined by appropriate platings. Homozygosity for sex in positive isolates was not determined. The analysis of one exceptional sectored colony has been omitted from the data. Isolates from both positive and negative sectors of this colony were uracil and tryptophan requiring and of sex *A*. Cell size suggested that they were not haploids but no successful matings were accomplished.

This suggests an exactness of exchange unexpected of radiation-induced chromatid breakage.

Induced meiosis with restoration of the diploid condition by fusion has seemed unlikely as the principal cause of induced homozygosis (JAMES 1955), and the following considerations provide further evidence against such a mechanism. If haploidy with later fusion occurred, then all heterozygous loci unlinked to sex would have an equal opportunity to effect an exchange. Under these circumstances it can be expected that homozygosis will be found at any such locus in the progeny of half the affected cells. A suitable sample of affected cells with which to test this hypothesis is available in galactose negative variant clones, providing the loci to be surveyed are unlinked to those for either galactose or sex. These requirements are fulfilled by the tryptophan locus in g_1g_1 variants (see table one). The frequency of *tr*/*tr* genotypes within g_1g_1 variant isolates is shown in table 3; only nine of 478 isolates were homozygous for tryptophan. Evidence against a hypothesis of meiosis is also provided by the data of table 3 on frequencies of homozygotes for uracil and sex. Recessive homozygosity for uracil, whose locus is independent of those for both sex and galactose, was found in only 20 isolates, and homozygosity for sex, unexpected under a hypothesis of meiosis and fusion, was found in 30 (20 *AA*, 10 *aa*) of the isolates. Thus, haploid formation plays a negligible part, if any, in the radiation-induced segregations under study here.

Evidence concerning the relative importance of mitotic crossing over as contrasted with first meiotic division can be obtained by a comparison of the characteristic frequencies of induced homozygosis at the loci for uracil, sex, and tryptophan. The information from such a comparison is not likely to be misleading unless the chromosomes involved are affected differently by radiation. In the case of mitotic crossing over one would expect a positive correlation between frequency of induced homozygosis and distance from centromere; in the case of first meiotic division a negative correlation (if crossovers occur) or no correlation (in the absence of crossing over) would be expected. The locations of the three loci in order of increasing proxim-

ity to their centromeres, are *Ur*, *A*, and *Tr*, as implied by proportions of tetratypes in G_2-*Ur*, G_2-*A*, and G_2-*Tr* segregations (data of table one). The data of table 3 show that the frequencies of homozygotes among galactose negative isolates were 20 *ur ur*, 20 *AA*, 10 *aa*, and 9 *trtr*. If the information from positive sectors is included, then the frequencies of recessive homozygotes for uracil and tryptophan (31 *urur*, 14 *trtr*) differ significantly. There is thus a positive correlation between frequency of induced homozygosis and distance from centromere, a fact which suggests that mitotic crossing over, rather than first meiotic division, is the mechanism by which the segregants are produced.

SUMMARY

Ultraviolet-induced chromosomal segregations in vegetative cells of diploid *Saccharomyces cerevisiae* have been indicated by a study of the behaviour of linked and unlinked marker genes in the formation of induced homozygotes for galactose utilization. The relative importance of (1) reciprocal translocation, (2) induced meiosis with subsequent fusion of haploid products, (3) first meiotic division, and (4) mitotic crossing over has been estimated. Results have suggested that mitotic crossing over is the mechanism by which the segregants are produced.

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GENOTYPIC BACKGROUND AND HETEROSIS IN DROSOPHILA PSEUDOOBSCURA[1]

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NATURAL populations of many species of Drosophila vary with respect to the gene arrangement in their chromosomes. In *Drosophila pseudoobscura*, at least sixteen gene arrangements are known in the third chromosome; these arrangements differ from each other by inversions of blocks of genes. The chromosome polymorphism in this species is balanced, i.e., the inversion heterozygotes have net selective advantages over the homozygotes. This selective advantage, or heterosis, has been shown to exist both in natural populations and in experimental populations bred in laboratories (DOBZHANSKY 1943, 1947b; WRIGHT and DOBZHANSKY 1946; DOBZHANSKY and LEVENE 1948).

The genetic mechanisms which bring about the heterosis in the inversion heterozygotes need elucidation. That this heterosis is delicately balanced has been shown in a variety of ways. In the first place it depends upon the environment in which the flies live. The carriers of a certain chromosomal constitution may be either adaptively superior or inferior to the carriers of other chromosomal constitutions depending upon temperature, nutrition, and on the presence or absence in the same population of other genotypic variants (DA CUNHA 1951; DOBZHANSKY and SPASSKY 1954; LEVENE, PAVLOVSKY, and DOBZHANSKY 1954). More important still, the adaptive value of a karyotype in a given environment depends upon the geographic origin of the constituent chromosomes. When experimental populations contain chromosomes derived from the same natural population, the inversion heterozygotes are often heterotic. However, in experimental populations of mixed geographic origin heterosis may be absent (DOBZHANSKY 1949). DOBZHANSKY and LEVENE (1951) reported that on hybridizing a chromosome type known as Standard (ST) from California with one known as Chiricahua (CH) from Mexico, they first observed a tendency toward elimination of the CH chromosomes, but then a change in the process and the final establishment of equilibrium of the two types. This was the first occurrence of the development of heterosis during the course of an experiment. They also reported that in a different population cage, using Arrowhead (AR) from California against CH from Mexico, they found equilibrium with no indication in this case that the heterosis had developed during the course of the experiment.

The origin of heterosis in experimental populations of mixed geographic origin has been studied further by DOBZHANSKY and PAVLOVSKY (1953), who found that the results of replicate experiments of this kind are not wholly determinate. In some populations containing ST chromosomes from California and CH from Mexico the structural heterozygotes have become heterotic, while in other populations of the

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same kind, and kept in similar environments, CH chromosomes were eliminated. This raised the problem whether the superiority of the inversion heterozygotes over the homozygotes is due to the genes in only the third chromosomes, or to a structuring of the whole genotype. The experiments described in the present report were designed to obtain information bearing on the above problem.

MATERIAL AND METHODS

All experiments were made with 12 strains of *D. pseudoobscura* derived from wild progenitors collected at Pinon Flats, Mount San Jacinto, California, and 12 strains collected at Santa Barbara, Chihuahua, Mexico. The collections in California were made by DR. D. F. MITCHELL in 1949, and in Mexico by PROF. H. T. SPIETH in 1947. These are the same strains which were used in the work of DOBZHANSKY and PAVLOVSKY (1953).

The experimental populations were maintained in population cages of a construction somewhat different from that used in the previous investigations. The cages used in the present experiments are lucite boxes, rectangular in shape, 4½″ high, 5¼″ wide and 18″ long. In the floor of the cage are 16 holes for the insertion of food vials. Since a fresh vial is inserted three times a week, each vial remains in the cage for a period of 37 days. The generation time is estimated at 25 days. All experimental populations were kept in constant temperature rooms at 25°C. The populations were sampled once a month for the first three months and every two months thereafter. Each sample consisted of 300 chromosomes, obtained from 150 larvae, taken in 6 subsamples on six successive days, 25 larvae (50 chromosomes) per subsample. The chromosome frequencies in tables 1–4 are, thus, based on 300 observations each.

CHROMOSOME TRANSFER BETWEEN GEOGRAPHIC POPULATIONS

In order to study the effect of background genotype on heterosis it was first necessary to obtain strains in which the third chromosomes of Californian origin were transferred onto the genetic background of Mexican origin, and vice versa. This had to be done in two stages.

First, third chromosomes marked with visible dominant mutant genes, and carrying gene arrangements other than those to be used in the experiments, were crossed repeatedly to the Californian and Mexican stocks. The marked chromosome in one case had the mutants, *orange*, *Blade*, *Scute*, and *purple* (*or BL Sc pr*) and had the Standard (ST) gene arrangement. In the other series, the chromosome was marked by the mutants, *Lobed* and *orange* (*L or*) and had the Santa Cruz (SC) gene arrangement. Strains bearing each of these marked chromosomes were crossed seven times both to the Californian (C) stocks which contained the gene arrangement Arrowhead (AR) and to the Mexican (M) stocks which contained the gene arrangement Chiricahua (CH). This procedure is shown in figure 1. As this was done with both markers for the Mexican as well as the Californian stocks, there were obtained four types of stocks containing mixed chromosomes with gene markers, as shown in figure 2.

In making the above crosses, the strains with the chromosomes containing the mutant markers were outcrossed each time to a different wild strain of either the

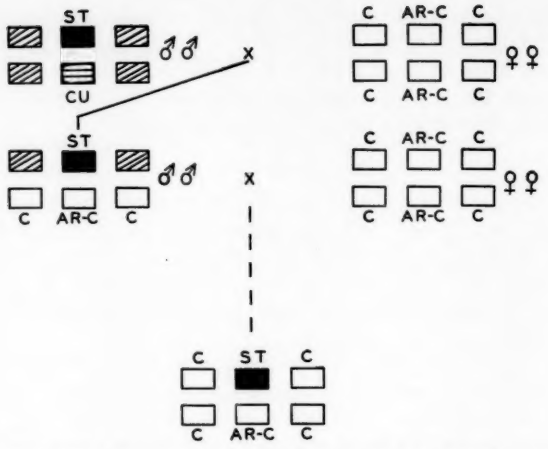

FIGURE 1.—Stage one of chromosome transfer between geographic populations. ST—Standard gene arrangement containing *or Bl Sc pr* mutants, CU—Cuernavaca gene arrangement containing recessive lethal mutant, AR-Arrowhead gene arrangement, C—California chromosomes. Cross-hatched chromosomes represent background chromosomes derived from diverse laboratory stocks. Broken lines represent five backcrosses of marker-chromosomes, with ST gene arrangement, to Arrowhead California stocks to provide an all California genotype except for marker-chromosomes.

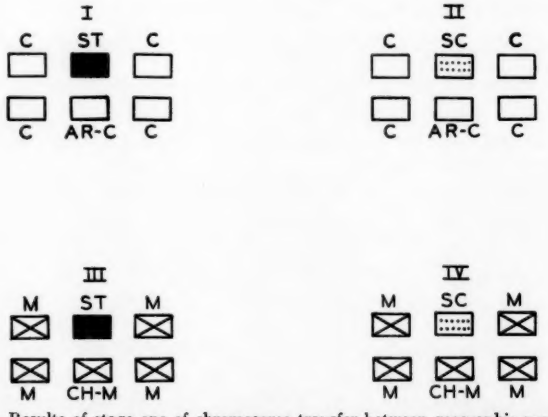

FIGURE 2.—Results of stage one of chromosome transfer between geographic populations. ST—Standard gene arrangement containing *or Bl Sc pr* mutants, AR—Arrowhead gene arrangement, C—California chromosomes, SC—Santa Cruz gene arrangement containing L or mutants, M—Mexican chromosomes, CH—Chiricahua gene arrangement. Represented are four marker-stocks containing visible mutants on either all California or all Mexican genotypes.

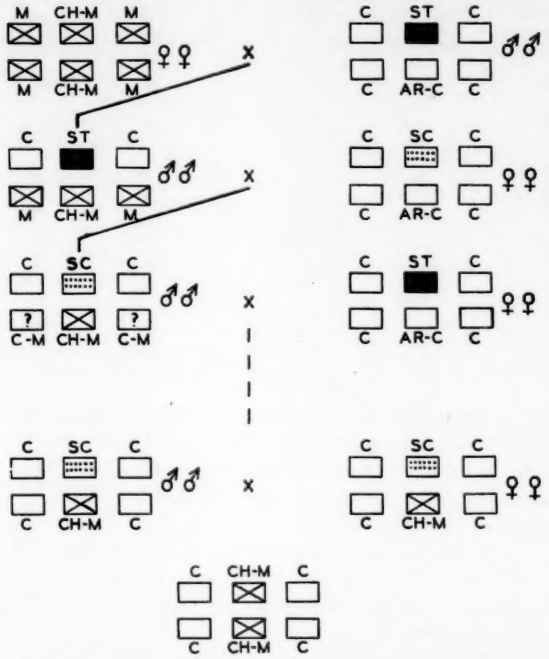

FIGURE 3.—Stage two of chromosome transfer between geographic populations. Symbols as on previous figure. Broken lines represent four more backcrosses alternately to the two marker-stocks which resulted in the experimental stocks which contained third chromosomes of one geographic origin and the remaining genotype of a different geographic origin.

Californian or Mexican origin. In this manner, strains were obtained which had the mutant markers but which also carried a variety of genetic modifiers which were present in the geographical populations used.

The second series of crosses is represented schematically in figure 3. The purpose of these crosses was to obtain strains which had the third chromosomes of one geographic origin and chromosomes other than the third of a different geographic origin. In *Drosophila pseudoobscura* the variations in gene arrangement are found mainly in the third chromosome (DOBZHANSKY and EPLING 1944), the chromosomes other than the third have usually the same gene arrangement regardless of geographic origin. For this reason, the transfers of Californian third chromosomes onto Mexico genetic background, and vice versa, had to be done by repeated backcrossing to the strains of the proper geographic origin. Seven such backcrosses were made. This means that less than 5% undesirable background chromosomes remained in the strains finally obtained.

Male flies were used whenever mixed background chromosomes were involved in

TABLE 1

Control experiments. Experimental populations containing CH and AR chromosomes of Californian origin

Month	Percent CH	
	Population D-1	Population D-2
0	50.0	50.0
1	51.1	45.0
2	41.3	35.7
3	35.7	38.3
5	39.0	34.3
7	33.7	33.7
9	28.7	29.7
11	30.3	39.3
13	27.3	28.3

order to prevent crossing over from contaminating the stocks. Third chromosome crossing over in the marker-stocks was eliminated by the constant maintenance of the marker-stocks as inversion heterozygotes. This has been shown to be a very effective crossover suppressor by DOBZHANSKY and EPLING (1948).

After obtaining the two types of third chromosomes on foreign backgrounds it was possible to place the two inversion types in competition with one another, in population cages, under any background chromosome situation. This was done by hybridizing the obtained strains with the original pure AR(C) or CH(M) strains maintained in our laboratory.

CONTROL EXPERIMENTS

Inversion heterozygotes which carry chromosomes with different gene arrangements, but derived from the same locality, usually exhibit heterosis. Thus DOBZHANSKY (1948) has shown that populations which contain AR and CH chromosomes of Pinon Flats origin reach, at 25°C, an equilibrium at about 75% AR and 25% CH. However, as stated above, it is known that the adaptive values of the heterozygous and homozygous karyotypes are exceedingly sensitive to modification by environmental agencies such as temperature, nutrition, and probably others. The experiments reported in the present paper were carried out under conditions appreciably different from those of DOBZHANSKY (1948). This was the result of the use of the plastic population cage, instead of the standard wooden model. The food vials have very different surface-volume ratios in the two types of cages with a result that the plastic cage supports an adult population which is appreciably smaller than the standard cage. Two control experiments were accordingly arranged in which the initial populations consisted of F_1 hybrids between strains homozygous for AR and for CH of Pinon Flats origin.

The results are summarized in table 1 and figure 4. Both control populations reached equilibria at about 70% AR and 30% CH. The changes in the relative frequencies of the chromosomal types observed in the two experiments were parallel, the composition of both populations remaining alike at all stages of the experiment

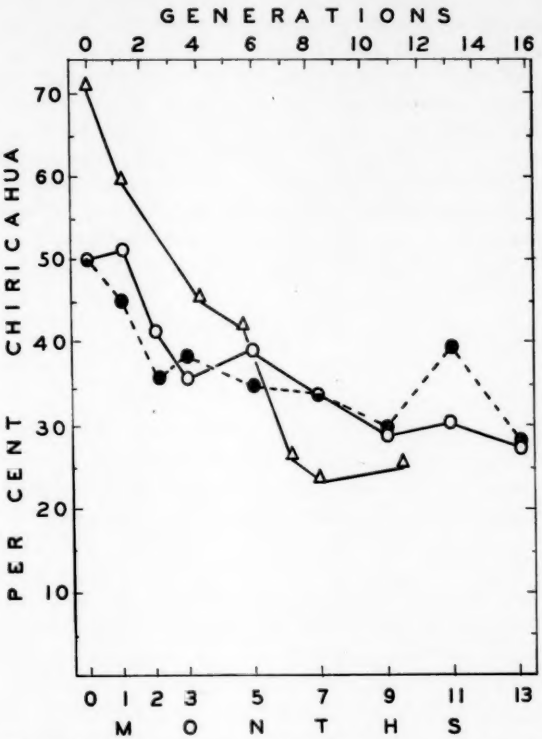

FIGURE 4.—Frequencies of chromosomes with the CH(C) gene arrangement in populations of geographically uniform origin. Population D-1—white circles, D-2—black circles, DOBZHANSKY's 1948 data—white triangles.

within limits of sampling errors. The aggregate chi-square for the 8 pairs of samples is 11.56, which corresponds to a probability of 0.2–0.1. The greatest divergence was observed between the samples taken in the replicate experiments during the eleventh month (table 1); such a divergence or a greater one has, however, a probability of chance occurrence between 0.05 and 0.02. Figure 4 gives a graphic comparison of the outcomes of the two control experiments with that of DOBZHANSKY (1948). No appreciable differences between the experiments are apparent, although the equilibrium level in the populations described by DOBZHANSKY may be slightly lower than in the present ones. If this difference is real, it may be accounted for by the dissimilarities of the environments of the two types of cages used in these experiments.

In attempting to estimate the adaptive values of the karyotypes it was found that both the method worked out by SEWALL WRIGHT, which assumes constant selective coefficients throughout the experiment, and that of HOWARD LEVENE, which permits

changing selective coefficients but in a regular fashion, give anomalous results (WRIGHT and DOBZHANSKY 1946; DOBZHANSKY and LEVENE 1951). An inspection of the data will show that there were changes in direction of selection that were both sudden and reversible. They were probably due to peculiarities of the type of cage used. This aspect of the experiment will be discussed later. As it is felt that these sudden and reversible changes in selection do not mirror the selection process it was decided to omit the obviously aberrant points and then calculate the adaptive values of the karyotypes using Wright's method. The data omitted were month #1 of population D-1 and months #2 and #11 of population D-2. The adaptive values of the karyotypes of these populations and those found by DOBZHANSKY (1948) are given below:

Karyotype	*Present experiments*	*Dobzhansky's experiments*
AR/AR	0.81	0.86
AR/CH	1.00	1.00
CH/CH	0.53	0.48

POPULATIONS WITH CALIFORNIAN GENETIC BACKGROUND

Strains with CH third chromosomes of Mexican origin and with other chromosomes mostly of Californian origin, were obtained as described above. These strains were crossed to others of pure Californian descent, but homozygous for the AR gene arrangement in the third chromosomes. Two replicate experimental populations were made using the F_1 hybrid flies. In these populations the genotype is, then, mostly derived from the Californian race, except that the third chromosomes are CH from Mexico and AR from California. The changes observed in these populations are summarized in table 2 and figure 5.

Both populations eventually reached equilibria at about the levels 70–75% AR and 25–30% CH, which is close enough to the equilibrium observed in the control

TABLE 2

Populations containing AR chromosomes of Californian origin, CH chromosomes of Mexican origin, and Californian genetic background

Month	Percent CH	
	Population A-1	Population A-2
0	50.0	50.0
1	43.3	43.7
2	41.7	32.3
3	47.7	38.3
5	32.3	30.7
7	34.7	29.0
9	39.0	31.7
11	21.7	23.0
13	21.3	19.7
15	23.3	27.0
17	25.0	31.7
19	22.0	27.3

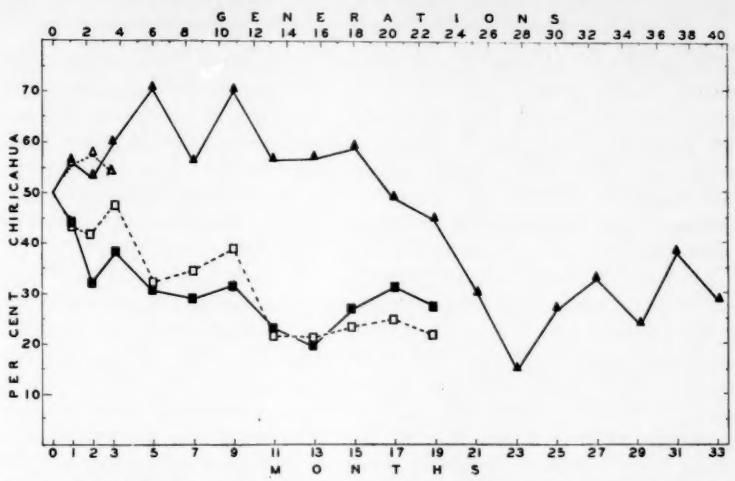

FIGURE 5.—Frequencies of chromosomes with the CH(M) gene arrangement in populations containing California genetic background: population A-1—white square, A-2—black squares; and populations containing Mexican genetic background: population B-1—white triangle, B-2—black triangle.

experiments (fig. 4). However, the frequency of CH chromosomes in the population A-1 decreased at first more slowly than in A-2, although the final equilibrium in A-1 seems even lower for CH than in A-2 (table 2 and fig. 5). The differences between the two populations are statistically significant in the samples taken during the second and third months (chi-squares 5.61 and 5.33 respectively, corresponding to probabilities around 0.02). Although in later samples the differences are no longer significant, the aggregate chi-square is 21.59, which, for 11 degrees of freedom, corresponds to a probability between 0.02 and 0.01.

Inspection of figure 5 suggests that the course of selection in the two populations under consideration is, on the whole, less regular and uniform than in the control populations (fig. 4). A sharp drop in the frequency of CH chromosomes between the ninth and eleventh months seems especially striking. The reason for this drop is not clear. It may have been caused by a sudden change in the genetic composition of the populations themselves, if a highly fit AR homozygote arose at that time. However, since a sharp drop in the percentage of CH(M) occurred in many of the other cages at this time, it is felt that the causative agent may have been environment. The drop coincided with a spell of very hot weather. Although the population cages were kept in a constant temperature room at 25°C the hot spell resulted in an increase of humidity in the constant temperature room and in a lush growth of molds in the cages. However, in spite of the irregularities noted in the selection process, it is apparent that in all populations with Californian background, AR from California and CH from Mexico form a heterotic combination.

POPULATIONS WITH GEOGRAPHICALLY MIXED GENETIC BACKGROUNDS

The parents of the experimental populations reported in this section were F_1 hybrids between strains in which the third chromosomes with CH gene arrangements of Mexican origin were transferred onto Californian genetic background, and strains with AR third chromosomes of Californian origin transferred to the Mexican genetic background. The resulting populations had, then, Mexican CH and Californian AR third chromosomes, and a great variety of genetic backgrounds resulting from assortment and recombination of Californian and Mexican chromosomes. The events observed in these populations are reported in table 3 and figure 6.

In contrast to the experiments previously described, the frequencies of the AR and CH chromosomes fluctuated in both populations around the starting frequency for more than 15 generations, after which the populations were terminated. The two replicate populations remained rather similar throughout (the aggregate chi-square is 11.37, corresponding to a probability between 0.1 and 0.2). What is more important is that the final frequencies of the AR and CH chromosomes were not appreciably different from the initial ones. This result was unexpected for two reasons. First it differs from the outcomes of the control experiments and of the experiments with the Californian genetic background. Secondly, it differs from the results observed by DOBZHANSKY and LEVENE (1951) in a population which had, like the present ones, Californian AR and Mexican CH third chromosomes and a geographically mixed genetic background. In the populations studied by DOBZHANSKY and LEVENE, the initial frequency of AR and CH chromosomes were, like in this study, 50%, but the final equilibrium was in the vicinity of 75% AR and 25% CH.

Two new populations were, accordingly, started, both with a mixed Californian-Mexican genetic background, and Californian AR and Mexican CH third chromosomes. However, in one of these new populations the initial frequencies were 20% CH and 80% AR (population C-3), and in the other 80% CH and 20% AR (popula-

TABLE 3

Populations containing AR chromosomes of Californian origin, CH chromosomes of Mexican origin, and geographically mixed genetic backgrounds

Month	Percent CH			
	Population C-1	Population C-2	Population C-3	Population C-4
0	50.0	50.0	20.0	80.0
1	46.3	46.0	20.0	64.3
2	49.3	44.3	23.3	68.7
3	54.3	49.3	21.7	65.7
5	48.3	52.0	27.7	49.0
7	48.3	44.3	24.0	43.0
9	51.7	55.0	29.0	43.3
11	50.7	43.7	25.7	35.7
13	42.3	49.3	26.0	25.0
15	—	—	—	31.3
17	—	—	—	37.0
19	—	—	—	26.0

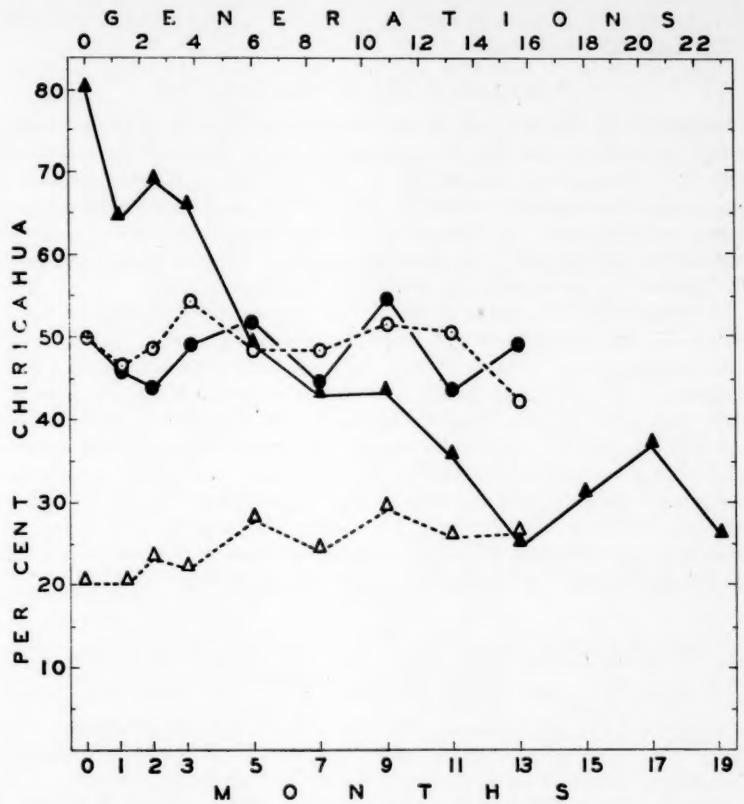

FIGURE 6.—Frequencies of chromosomes with the CH(M) gene arrangement in populations of geographically mixed genetic backgrounds. Population C-1—white circles, C-2—black circles, C-3—white triangles, C-4—black triangles.

tion C-4). In populations in which all chromosomes are of uniform geographic origin, the initial frequencies of the competing gene arrangements do not affect the final outcome. Identical or very similar equilibrium frequencies are established regardless of the initial frequencies of the chromosomes (DOBZHANSKY 1947a, 1948). The results observed in the populations C-3 and C-4 are also reported in table 3 and figure 6.

The behavior of the populations C-3 and C-4 is in agreement with that of the population described by DOBZHANSKY and LEVENE (1951), and not with that of populations C-1 and C-2. In C-4 the frequency of CH chromosomes dropped sharply, and approached an equilibrium at a level between 25 and 30 percent. In C-3 there was a slight but apparently significant increase in the frequency of CH chromosomes,

up to the same equilibrium level as in C-4. Possible interpretations of these results will be discussed below.

Populations with Mexican genetic background

Populations of this kind gave the most complex and interesting results. As many as eight of them were studied, two populations having been kept for almost three years (forty generations), with periodic samples being taken. In every instance, the initial populations consisted of hybrids obtained by crossing strains of pure Mexican origin, homozygous for CH, with strains having AR chromosomes of Californian origin which had previously been transferred onto the Mexican genetic background. The observations are summarized in table 4 and figures 5 and 7.

Two populations, B-1 and B-2, (fig. 5) had the initial frequencies of the CH and AR chromosomes 50% each. B-1 was started earlier than B-2, and was kept for three months, during which it showed no appreciable changes in the karyotype composition. This seemed to parallel the behavior of the populations C-1 and C-2, which had geographically mixed backgrounds (see above). However, the later replicate, B-2, showed a significant, though very erratic, increase in the frequency of CH chromosomes, which reached the 70% level after 5 and after 9 months. However, instead of establishing and maintaining an equilibrium, the CH chromosomes began to dwindle in frequency after 15 months. The frequency of CH reached a value as low as 15% after 23 months, only to rise again to 30% two months later and fluctuated around that point for the duration of the cage.

TABLE 4

Populations containing AR chromosomes of Californian origin, CH chromosomes of Mexican origin, and Mexican genetic background

Month	Percent CH							
	Population B-1	Population B-2	Population B-3	Population B-5	Population B-7	Population B-4	Population B-6	Population B-8
0	50.0	50.0	20.0	20.0	20.0	80.0	80.0	80.0
1	55.3	56.3	24.3	15.0	16.7	74.0	60.3	60.0
2	57.3	53.0	31.7	17.7	10.3	68.3	59.3	64.7
3	53.7	60.0	28.7	22.7	16.0	62.7	40.3	50.7
5	—	70.3	31.7	36.7	21.3	59.0	42.7	52.0
7	—	56.0	28.7	36.7	13.7	49.7	41.3	44.0
9	—	70.0	29.0	33.0	18.7	49.0	31.7	34.0
11	—	56.3	29.3	24.0	—	64.0	32.7	42.0
13	—	56.7	—	20.0	—	53.0	36.3	—
15		59.0	—	47.3	—	54.7	47.0	—
17		49.0	—	24.0	—	29.3	32.3	—
19		44.7	—	39.0	—	27.3	34.0	—
21		31.0	—	—	—	34.7	—	—
23		14.7	—	—	—	39.3	—	—
25		27.0				40.3		
27		33.0				33.0		
29		24.0				31.7		
31		38.3				42.0		
33		28.7						

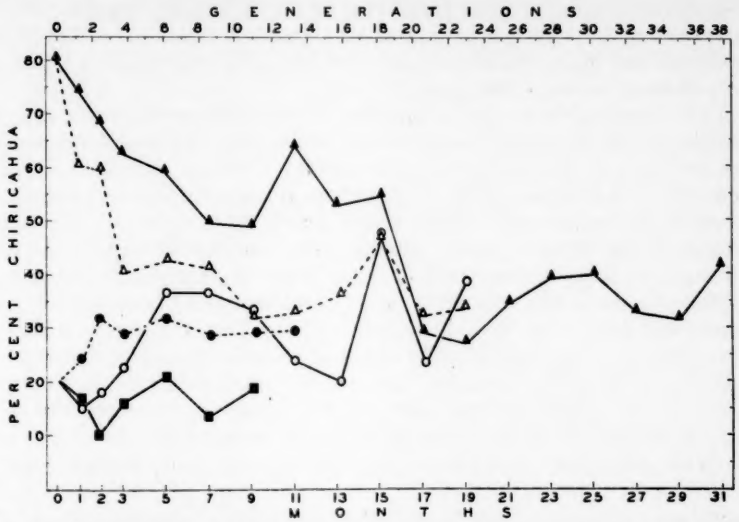

FIGURE 7.—Frequencies of chromosomes with the CH(M) gene arrangement in populations containing Mexican genetic background. Population B-3—black circle, B-4—black triangle, B-5—white circle, B-6—white triangle, B-7—black square.

Populations of similarly mixed geographic origin, but with different initial frequencies of AR and CH chromosomes, behaved quite differently. Thus, the populations B-3 and B-5 (fig. 7) were started at different times, to be sure, but both with initial frequencies of 20% CH and 80% AR chromosomes. Population B-3 reached a level of about 30% CH two months after the start. This level was preserved without significant fluctuations, as expected when a true equilibrium has become established. This was not so in population B-5. Here there was at first no change in the karyotype composition, or even a drop in the frequency of CH to the 15% level. But this trend (if it was real) became reversed, and CH rose to the 35% level and remained there from the fifth to the ninth months. Thereafter the cage behaved very erratically. There was a rather sharp drop in CH frequency to 20% by the thirteenth month, and a sudden jump to about 50% at the fifteenth month. This was followed by a drop to 24% CH and a rise to 39% CH at the next two readings.

The replicate, though not simultaneous, populations B-4 and B-6 (fig. 7) had initially 80% CH and 20% AR. In both populations the frequency of CH chromosomes dropped. However, while in B-6 the drop was reasonably (though not entirely) regular, population B-4 showed great oscillations. The frequency of CH, in B-4, at first dropped gradually, and seemed to establish an equilibrium at about 50% (with a significant jump 11 months from the start). Then after 15 months there occurred a sudden and dramatic drop to 30% and a recovery to about 40% when the population was 2 years old. Subsequently there was a return to the 30% level when the population was 29 months old and another rise to 42% CH two months later. The sudden and dramatic drop in CH frequency in B-4, noted above, coincided in time

with sharp decreases in CH frequency in other populations (for example, the drop at the twenty-first month in B-2 and at the eleventh month in A-1 and A-2). The possibility that these coincident changes were caused by environmental factors has been discussed earlier in this paper.

Two phenomena observed in the experiments on populations with mixed and with Mexican genetic backgrounds would be very unusual in populations of uniform geographic origin, at least under reasonably carefully controlled environmental conditions. These phenomena are the establishment of somewhat different equilibrium levels in populations with initially similar genetic components, and the erratic progress of the selection process, especially with reversals of trends. Such phenomena have, however, been described in populations of geographically mixed origin by DOBZHANSKY and LEVENE (1951) and DOBZHANSKY and PAVLOVSKY (1953 and unpublished data). They have also been recorded by EPLING, MITCHELL and MATTONI (1953), although not ascribed by these authors to the geographic mixtures.

However, before the observations here described can be put in the same category as those of the authors just cited, other possibilities must be considered. One possibility is the effect of the use of the plastic population cages, with their populations which are numerically smaller than those obtained in the larger standard cages. It seemed barely conceivable (although not probable) that the fluctuations in the chromosome frequencies might be caused by genetic drift. Populations B-7 and B-8 were, accordingly, started and put in standard wooden cages, with Mexican CH and Californian AR chromosomes on Mexican genetic background. Population B-7 had initially 20% CH chromosomes, and thus was a replicate of B-3 and B-5; population B-8 had 80% CH chromosomes, and constituted a replication of B-4 and B-6 (table 4 and fig. 7; B-8 is not shown in graph form).

Population B-7 fluctuated between 10% and 20% CH for 9 months and was then terminated. Its mean value was about 15% CH. This population B-7, showed fluctuations about the equilibrium level but none so violent as those observed in most of the plastic cages. Cage B-8 has shown a fall in CH frequency from the initial value of 80% to about 40%. It is too early to tell whether or at what value it will reach equilibrium. It seems possible from the results in B-7 that the standard wooden cages give fewer irregular fluctuations than the plastic cages. This probem will be discussed below.

Another possible explanation of these inconsistent results concerns itself with the method of transfer of the third chromosomes to the various genetic backgrounds. Since it is impossible to be certain that the background genotype is entirely of Californian or of Mexican origin, one must rely on repeated backcrosses to eliminate the undesired background chromosomes. It is, then, possible that some chromosome sections of Californian origin were present in the populations and that there was selection for these Californian elements. This possibility seems to have some substantiation in the fact that both the early populations, which were maintained for long periods of time, and the later populations gave results indistinguishable from the populations with Californian genetic background. One also finds that the later B type populations do not show the initial plateau of 50–55% CH that the earlier populations showed.

TABLE 5

Initial dates of experiments

Population	Initial Date
D-1	10/1/52
D-2	10/5/52
A-1	10/10/52
A-2	10/15/52
C-1	1/10/52
C-2	12/15/51
C-3	3/10/53
C-4	3/10/53
B-1	10/10/51
B-2	12/5/51
B-3	4/5/52
B-5	3/20/53
B-7	11/15/53
B-4	4/10/52
B-6	3/20/53
B-8	11/15/53

DISCUSSION

The only common denominator in all the experimental populations described in the present article is that in every one of them the two kinds of chromosomes, AR and CH, tend to persist indefinitely. In no population has either chromosomal type come close to being eliminated. This result is what is expected if the Californian AR and Mexican CH chromosomes produce heterosis, i.e., make the heterozygous karyotype, AR/CH, superior in adaptive value to both homozygotes, AR/AR and CH/CH. Such heterosis could not have been predicted. To be sure, AR/CH heterozygotes were known to be heterotic in at least some environments, provided that both chromosomes are derived from the same geographic population, as in the control experiment reported in table 1 and figure 4. However, populations having ST chromosomes from California and CH from Mexico may have the latter chromosomes eliminated (DOBZHANSKY and LEVENE 1951; DOBZHANSKY and PAVLOVSKY 1953).

Beyond that, the behavior of a population is influenced by the geographic origin not only of the third chromosomes, which are being followed cytologically because they are visibly different under the microscope, but also the remainder of the karyotype, which is geographically not differentiated as to the gene arrangement. Indeed, the populations reported upon in figures 5, 6, and 7 all had Californian AR and Mexican CH chromosomes; the differences between these populations concerned the geographic origin of the rest of the karyotype, which was either Californian, or Mexican, or of mixed origin. The events in a population are determined by the degree to which the heterozygotes are superior to the homozygotes, as well as by whether the two homozygotes are equal in selective value or whether one of them is superior to the other. The relative magnitudes of the selective values have been known to depend on environmental agencies such as temperature, nutrition, and presence of other genotypes in the same medium (DA CUNHA 1951; DOBZHANSKY and

SPASSKY 1954; LEVENE, PAVLOVSKY, and DOBZHANSKY 1954). The present study shows them to be determined not only by the genes in the chromosome pair directly concerned but by the genotype as a whole.

These two relationships, the fact of heterosis and that of the influence of the genetic background, proved to be unexpectedly obscured by another phenomenon. This is a striking indeterminacy in the behavior of populations that are replicates, at least in the sense of having the same initial components. Wide divergences in the outcomes of what should have been replicate experiments can easily be seen in figures 5–7. A closer examination shows that the divergences are of at least three kinds; (1) irregular fluctuations in the frequencies of a given class of chromosomes which exceed the expected sampling errors, (2) different equilibria apparently reached by populations of qualitatively or even quantitatively similar initial composition, and (3) erratic selection trends, which make the same genetic component of a population alternately increase and decrease in frequency.

The first kind of fluctuations may be due to several possible causes. That these fluctuations seem to occur mostly in the small lucite population cages used in these experiments, and rarely if at all in the larger cages appears to be significant. The low and fluctuating numbers of adult flies in the small cages may permit some fluctuations caused by genetic drift. A more likely possibility, suggested by the work of BIRCH (unpublished) is that the adaptive values of the karyotypes may be a function of the population density, and particularly of the presence or absence of competition for food among the larvae. Indeed, the experiments of BIRCH show some irregular fluctuations which are quite similar to those in the data reported in the present article. Expansions and contractions of the populations in the small cages have repeatedly been observed, especially in connection with such things as extreme weather conditions, development of molds, etc. Sharp but reversible changes in the selection pressures could easily be induced by such expansions and contractions.

The second and third kinds of fluctuations (changes in equilibrium values and in selection trends) have been observed in populations of mixed geographic origin, and rarely if ever in populations of geographically uniform origin (DOBZHANSKY and PAVLOVSKY 1953; DOBZHANSKY and LEVENE 1951). The explanation of these interesting but complex phenomena suggested by the authors referred to is, briefly, as follows. The gene complexes in the chromosomes with different gene arrangement from the same natural population have been coadapted by a long process of natural selection to produce superior fitness in heterozygotes. The results of WALLACE (1955) and VETUKHIV (1953, 1954) as well as those described in the present article suggest that the coadaptation involves not a single pair of chromosomes, but indeed, the whole genotype. Now, in artificial populations of uniform geographic origin the coadapted genotypic system is not altered qualitatively. All that occurs are changes in the relative frequencies of certain pre-existing gene complexes carried in the chromosomes with different gene arrangements. This is not so in populations of geographically mixed origin. Here the recombination of genes derived from different populations (races) gives a great variety of genotypes, many or most of which may have never existed in nature. A process of natural selection is, then, enacted in the artificial populations. New adaptively integrated genotypes are formed, and they

may be different in different populations. In a given population the selective process takes time, and different genotypes may gain prevalence at different stages of this process. The indeterminacy of the results is, then, a function of the field of potentially possible gene combinations being too great in relation to a limited size of the experimental populations.

In the experiments here described, the populations had one of the third chromosomes differ in origin from its partner as well as from the other chromosomes. Furthermore, in all instances care was taken to have several strains from each geographic region enter in the composition of each experimental population. Enough genetic variance was, thus, available (even disregarding the possibility of chromosomes or chromosome sections of other geographic origin slipping through the crosses shown in figures 1 and 3) to start a selective process of coadaptation between the gene contents of the different third chromosomes and of other chromosomes. Nevertheless, the AR/CH heterozygotes proved superior to the AR/AR and CH/CH homozygotes in all populations, as though these gene contents were coadapted to each other as they usually are when the chromosomes are of uniform geographic origin. Two possibilities may be mentioned as tentative explanations of this fact. The first is that, regardless of their geographic origins, AR and CH chromosomes interact to produce heterosis. This could happen if one of their gene arrangements first arose in a region where the other was also present, and the gene contents of these chromosomes were coadapted by natural selection. The coadaptedness of the Californian AR and Mexican CH chromosomes would, then, be a genetic relic of their former sympatric occurrence.

Another, perhaps more likely possibility, is suggested by the recent work of BRNCIC (1954), WALLACE (1955), and particularly LERNER (1954). BRNCIC and WALLACE found that juxtaposition of whole chromosomes of different geographic origins produces heterosis, which disappears when these chromosomes are broken by crossing over. LERNER has put forward the view that, at least in sexual and cross-fertilizing organisms, heterozygosis per se may be a viability stimulus. Now in the experimental populations described in the present article the third chromosomes of Californian and Mexican origins were prevented from exchanging segments because the inversion heterozygote, AR/CH, acts as a crossover suppressor (DOBZHANSKY and EPLING 1948). The inversion heterozygotes were thus necessarily heterozygous for alleles at many loci. The Californian and Mexican chromosomes with AR and CH gene arrangements carry gene complexes which have proven successful in their respective localities. As shown by BRNCIC and WALLACE, such heterozygosis results in heterosis. To be sure, the stimulating effects of allele heterozygosis may be offset by a lack of coadaptation between the gene contents of the third chromosomes and the rest of the genotype, as observed by DOBZHANSKY and PAVLOVSKY (1953) for ST chromosomes from California and CH chromosomes from Mexico. The fact that species hybrids are often inviable or sterile is conclusive evidence that heterozygosis per se does not guarantee superior fitness. Heterozygosis and coadaptation (or lack of coadaptation) may be regarded as two variables the interaction of which is responsible for the relative indeterminacy of the results of selection in the experimental populations described.

SUMMARY

Strains of *Drosophila pseudoobscura* were used which were derived from flies captured in California and which contained the Arrowhead (AR) gene arrangement, and other strains which came from Mexico and contained the Chiricahua (CH) gene arrangement in the third chromosomes. By a series of crosses, the AR chromosomes of Californian origin were transferred to the Mexican genetic background, and conversely, Mexican CH chromosomes were transferred to the Californian genetic background. Experimental populations were then formed, all of which had Californian AR and Mexican CH chromosomes; some of these populations contained, however, a Californian genetic background, others contained a Mexican genetic background, while still others contained a mixed genetic background of Californian and Mexican chromosomes.

All experimental populations remained chromosomally polymorphic, with AR and CH chromosomes eventually stabilized at certain equilibrium frequencies. The AR/CH heterozygotes were, thus, heterotic, i.e., superior in fitness to the AR/AR and CH/CH homozygotes. The position of the equilibrium level varied however, depending upon the geographic orgin of the genetic background, each gene arrangement being most frequent on its native genetic background. This second conclusion was partly obscured by the changing nature of the genetic background. Furthermore, the course of the selection process was often erratic and some of the replicate experiments produced significantly different outcomes.

The most probable interpretation of these observations is that although heterosis was formed under all the genetic backgrounds tested in these experiments, the level at which this equilibrium was achieved is a function of the genetic background of the population. The relative indeterminacy of the outcome in the experimental populations was due to the complex nature of the selective process which led to the establishment of novel adaptive genotypes from the genetic elements contributed by the hybridization of populations of different geographic origins.

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THE RELATIONSHIP OF CHIASMATA AND CROSSING OVER IN LILIUM FORMOSANUM

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THE origin of chiasmata has been a subject for controversy since JANSSENS (1909, 1924) proposed that they result from crossing over. Although the partial chiasmatype hypothesis has been widely adopted at various times and places, its general validity has recently either been questioned or not accepted in research reports (COOPER 1949; RHOADES 1946; STEINITZ-SEARS and SEARS 1953), in review articles (STURTEVANT 1951; HUGHES-SCHRADER 1952), and in an introductory text-book (SRB and OWEN 1952). The bearing of some of these demonstrations on the chiasmatype hypothesis will be considered in the discussion, as well as the alternative hypotheses of SAX and MATSUURA.

Evidence in favor of the chiasmatype hypothesis has been presented by many different workers and from a wide variety of different organisms. Some demonstrations point to a parallelism between genetic and cytological phenomena such as the similar effect of temperature differences on frequency of crossing over and of chiasma-formation; these indirect demonstrations have been summarized by MATHER (1938) and will not be considered in detail in this report.

A correspondence between the frequencies of chromosome associations and recombination of genetic markers has been shown in only one case, that of BEADLE'S (1932) Zea-Euchlaena hybrid in which 20 percent associations in a specific chromosome region led to the expectation of 10 percent crossing over, a value very close to the observed 12 percent crossing over. However, as emphasized again by COOPER (1949, p. 112) BEADLE was careful to point out that he did not have direct cytological evidence that the observed associations were really chiasmata.

Double interlocking of two bivalents has been considered an excellent demonstration of the chiasmatype hypothesis (MATHER 1938). However, only three examples have been offered in support of the hypothesis (two in Lilium, MATHER 1933; BEAL 1936, and one in Eremerus, UPCOTT 1936). Furthermore, as pointed out quite clearly by MATSUURA (1944), the critical chiasma has apparently been identified only by analogy. A single twist of the two homologues prior to pairing could result in a similar node. Until an accurate discrimination between these two possibilities has been made, this sort of demonstration can carry no weight as evidence.

Other types of cytological evidence are based on studies of multivalent configurations or structural heterozygotes. Certain single, intercalary chiasmata in quadrivalents and trivalents have been shown by DARLINGTON (1930) and DARLINGTON and MATHER (1932) to be inexplicable except as the consequence of genetic crossing over unless one assumes multi-strand, rather than 2-by-2 pairing at zygotene. A

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similar conclusion was reached by MATHER (1935) from observations of association of small fragments with normal bivalents in Lilium. In neither of the first two examples was it apparently possible for the workers to validate their interpretations through identification of exchange types during the subsequent anaphase, and their deductive conclusions have thus remained in need of such substantiation. In the case of the fragment chromosomes, however, such a validation should have been possible from a study of anaphase separations and the fact that the expected exchange types were not reported casts doubt on the interpretation of the observed associations as chiasmata.

In rings formed by translocation heterozygotes of 6 or more members, a chiasma between interstitial homologous sections of two otherwise non-homologous chromosomes will result in a "figure-of-eight" rather than an open ring configuration. According to DARLINGTON (1931), such an interstitial chiasma could result only from genetic exchange, and would provide an explanation for the origin of new types of rings in Oenothera. Numerous examples of interstitial chiasmata were later found by SANSOME (1932) in a translocation heterozygote of *Pisum sativum*, and she was able to identify the chromatids in several of them. A "figure-of-eight" configuration was found also in a complex translocation heterozygote of Datura by BERGNER and BLAKESLEE (1932) who were able to recover an expected crossover type. For Datura, no quantitative data were offered to enable a comparison of the frequency of interstitial chiasmata with that of certain crossover types among the progeny.

Heteromorphic bivalents have been observed in many different plants and animals but have been little used in support of the chiasmatype hypothesis. KOLLER (1938) reported a consistent relationship between chiasma frequency and equational separations in the heteromorphic sex chromosomes of the golden hamster. HAGA's (1944) analysis of a small terminal deficiency in Paris showed a significantly higher proportion of equational separations at anaphase than would have been expected from the observed number of chiasmata. More recently, BARTON (1951) found neither chiasmata nor crossing over in a heteromorphic example of the nucleolus-organizer arm of chromosome 2 of tomato. Evidence obtained from heteromorphic bivalents must be considered also with regard to the possibility of equational separation of the centromere, and this problem will be given further attention in the discussion.

Critical analyses of inversion heterozygotes have not been provided. DARLINGTON (1937) has diagrammed several of the theoretical expectations of crossing over in inversion heterozygotes. On the basis of the chiasmatype hypothesis, various types of asymmetric bivalents and characteristic "inversion" chiasmata would be expected at diakinesis and metaphase, but actual demonstrations of such configurations have been meagre.

In summation, the evidence in favor of the chiasmatype hypothesis is fragmentary and incomplete. It seems worthwhile, therefore, to re-examine the relation between chiasma formation and crossing over in an organism, such as Lilium, which readily permits the identification both of chiasmata and of the exchange types at anaphase. The present report provides information obtained from two terminal deficiencies and a paracentric inversion.

MATERIALS AND METHODS

Seed of *Lilium formosanum* was obtained from a commercial source, and the plants were grown in the greenhouse and field at Berkeley. The cultures show variation in easily noted vegetative characteristics such as vigor and anthocyanin pigmentation; a partially male-sterile condition, presumably due to a recessive gene, is appearing in some populations. Meiotic irregularities were found in two of the aberration types to be described later. However, routine examination of the pollen-mother cells of several hundred other plants has revealed only the usual meiotic sequence.

Structural heterozygotes were found among the progeny obtained after use of pollen treated with a 1000r dose of X-rays. Tetraploid individuals were produced by means of EMSWELLER and LUMSDEN's (1943) method of treating bulb-scales with colchicine.

Anthers were fixed for 24 hours or longer in the 3:1 alcohol-acetic mixture and their contents were examined in temporary aceto-carmine mounts. Because the anthers were quite large, they were cut into nine or ten sections before smearing. Usually only a few such sections were needed to give an adequate number of cells. The cells at the edge of the slide were obscured by the sealing compound and large clumps of cells were routinely excluded from the tallies; the totals for each anther section were much larger, therefore, than is apparent from the tabular entries. In a few cases the preparations were exceptionally good and only 3 or 4 unanalyzable cells occurred on each slide; these few cells were not tallied and are not listed in the tables. In several cases, when only two slides from adjacent anther sections were available, individual slide tallies were not made during the collection of the data.

STEWART's (1947) karyotype of the closely related species, *L. philippinense*, was used for identification of the chromosomes.

TERMINAL DEFICIENCIES

Chromosome A

This deficiency included about two thirds of the short arm and was apparently terminal since no chiasmata were observed which united the ends of the deficient and the normal short arms. The heteromorphic homologues always formed a bivalent with at least one chiasma in the long, normal arm. For the deficient arm, no configurations were observed which could not be explained as due either to the absence of a chiasma (fig. 1, 2) or to the presence of a single chiasma (fig. 3–5). At first anaphase, separations of the deficient arm could be readily classified as equational (fig. 7, 8) or reductional (fig. 6).

Table 1 presents the results of tallies made with the original X_1 plant and with an X_2 plant obtained by selfing the original plant. In both cases, it was possible to compare metaphase and first anaphase stages obtained from the same part of a single anther, and, in both cases, there was an excellent correspondence between the occurrence of chiasmata at metaphase I and equational separations at anaphase I. Three of the four heterogeneity tests showed good agreement of frequencies among the several slides examined. With respect to the significantly heterogeneous set (anaphase, table 1b), an observation made frequently during the course of this

TABLE 1

Chiasmata at metaphase I and segregation at anaphase I for a deficient short arm of chromosome A

a. Field grown X_1 plant (018-33), collected Sept. 7, 1951; five slides from the upper half of one anther

Slide no.	Metaphase		Anaphase		Not analyzable	
	Xma	No Xma	Eq.	Red.	Meta.	Ana.
1	100	40	33	11	2	6
2	51	26	35	11	15	8
3	74	28	30	13	20	7
4	74	35	73	33	7	18
5	126	54	1	1	5	0
Totals	452	183	172	69		
Heterogeneity						
χ^2	1.2		1.6			
df	4		4			
P	>0.80		>0.80			
χ^2, metaphase vs. anaphase						
totals	0.17					
df	1					
P	>0.50					

b. Field grown X_2 plant (018-33 selfed), collected Sept. 8, 1953; five slides from the upper half of one anther

1	34	37	9	4	3	1
2*	—	—	18	20	—	5
3	27	22	6	7	4	0
4	29	24	9	17	2	2
5	30	31	39	19	2	4
Totals	120	114	81	67		
Heterogeneity						
χ^2	1.0		10.2			
df	3		4			
P	>0.80		>0.02			
χ^2, metaphase vs. anaphase						
totals	0.42					
df	1					
P	>0.50					

* This slide was slightly overstained, and metaphase tallies were not made.

work is pertinent. It has repeatedly been noted that cells of one class; i.e., with or without a chiasma in a specific region, tend to occur in groups or relatively restricted regions on a slide. This sort of clumping effect would lead to a lack of homogeneity among several slides made from one anther if only a few cells of a specific stage were present on each slide. It is believed to be responsible for the high heterogeneity chi-square just mentioned. Since tallies for single slides with more cells of a given stage would already include heterogeneous groups of cells, it seemed reasonable to add together the anaphase tallies from these slides. If, however, a significant discrepancy between the totals for anaphase and metaphase had thereby resulted, the heterogeneity would have weakened the conclusion that a correspondence did not exist.

In the X_1 plant, the frequencies of chiasmata at metaphase I and of equational separations at anaphase I for the deficient arm were 70 and 71 percent, respectively, while in the X_2 plant, the respective values were 51 and 55 percent. When the frequencies of chiasmata at metaphase in the two plants were compared statistically, a χ^2 of 25.7 was obtained, with df = 1, and $P < 0.01$. The comparison for anaphase between the two plants was also highly significant, $\chi^2 = 11.2$, df = 1, and $P < 0.01$. It is thus of interest to note that the correspondence between frequency of chiasmata and equational separation is maintained for a specific chromosome region even though the frequency of both events is markedly different between the two plants. Since the two plants presumably differed genetically and were grown during different years, any or all of several factors may have been responsible for the difference in frequency.

Chromosome I

This deficiency included about two thirds of the long arm and was apparently terminal. The first collections available for comparison came from two field-grown X_1 subdivisions, and were obtained within 8 days at the peak of the summer growing season (table 2a). The second collection was made the following year from another X_1 subdivision but early in the growing season and shortly after the plants had been transplanted from the greenhouse to the field. Meiosis was normal in the material collected in 1952 but that collected in 1953 showed partial desynapsis with as many as six univalents present at late diakinesis or metaphase.

From the collections made in both years, the presence or absence of chiasmata in the deficient chromosome (fig. 9, 10) and the type of separation at first anaphase (fig. 11, 12) could be determined. For the 1952 material, only the early diakinesis stage was available for the investigation of chiasmata. At this stage it was not possible to identify the centromere so that the arm in which a single chiasma occurred had to be determined by the distance of the chiasma from either end of the chromosome. Measurements made from mitotic division figures gave a ratio of 4:1 for the length of the deficient long arm to that of the normal short arm. This ratio was then used as a basis for classifying the chiasmata according to the arm in which they occurred. In this fashion, all but 16 of 168 chiasmata (table 2a) were accurately located with the majority occurring at or near the end of either arm. In every case in which two chiasmata were observed, each was clearly in a different arm. The

TABLE 2

Chiasmata at diakinesis and segregation at anaphase I for a deficient long arm of chromosome I

a. Field grown X_1 subdivisions (018-118); diakinesis from subdiv. no. 6, collected Aug. 19, 1952; anaphase from subdiv. no. 4, collected Aug. 11, 1952

Tallies at early diakinesis (one slide)		Chiasma in deficient arm:	
		Present	Absent
One chiasma in deficient arm	121	121	
One chiasma in short arm	10		10
One chiasma in each arm	21	21	
No chiasma	3		3
One chiasma, location not determinable	16	*Corr. 8*	*8*
Unanalyzable cells	27		
Totals: Uncorr.	155	142	13
Corr.	*171*	*150*	*21*

*Tallies of type of anaphase I segregations from anaphase I and II (two slides each from different anthers of one flower)**

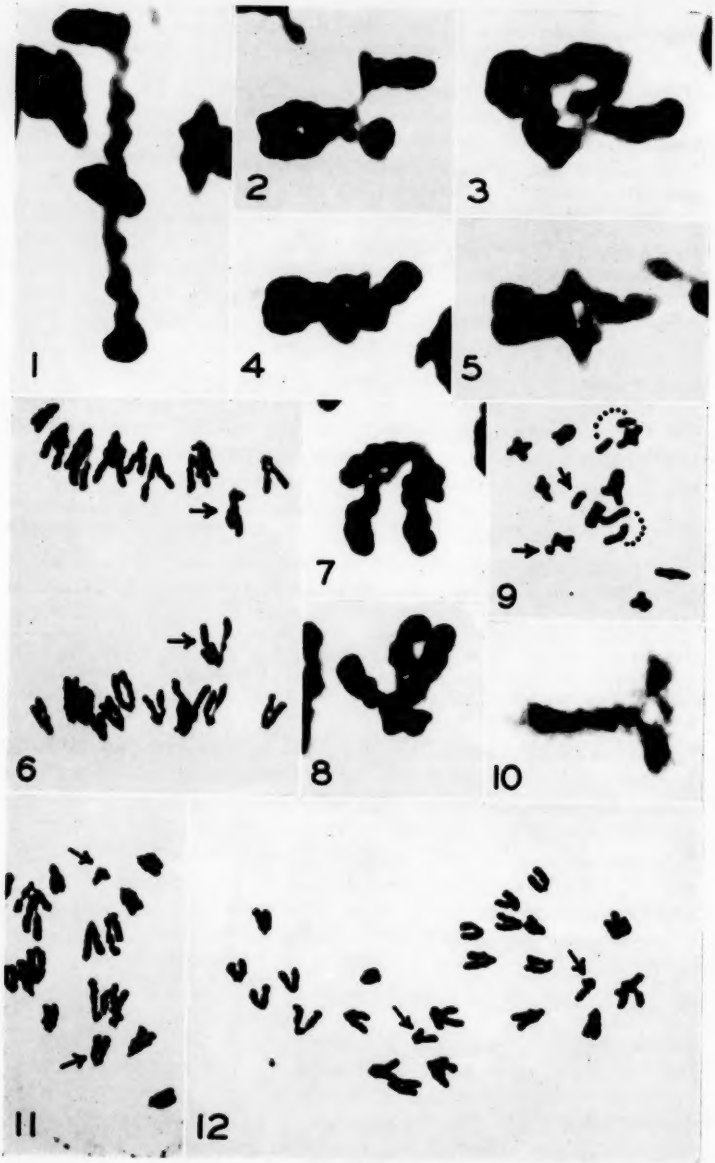
	Equational	Reductional
Anaphase I	90	10
Anaphase II (whole meiocytes)	33	5
Totals	123	15

χ^2, totals of diakinesis uncorr. vs. anaphase, = 0.27, df = 1, $P > 0.50$.
χ^2, totals of diakinesis corr. vs. anaphase, = 0.043, df = 1, $P > 0.80$.

*b. Field grown X_1 subdivision (018-118), collected June 26, 1953 (four slides from the lower third of one anther)**

Slide no.	Late diakinesis		Anaphase I	
	Xma	No Xma	Eq.	Red.
1	193	52	27	14
2	80	20	28	9
3	0	0	73	16
4	0	0	21	4
Totals	273	72	149	43
Heterogeneity				
χ^2	0.014		4.9	
df	1		3	
P	>0.90		>0.10	
χ^2, late diakinesis vs. anaphase totals	0.1			
df	1			
P	>0.70			

* Unanalyzable cells not tallied, see text.



FIGS. 1–12

16 chiasmata whose location could not be determined with sufficient accuracy to assign them to a specific arm were assumed to occur with equal frequency on either side of the centromere, and were thus apportioned as the corrected values in table 2a. Their inclusion in the totals obviously makes very little difference for the statistical comparison.

In the 1953 material, counts of chiasmata were made at late diakinesis, at a time when the centromere could be recognized, and only the chiasmata occurring in the deficient arm were tallied (table 2b). Observations on desynapsis in this material will be summarized in the next section.

For the 1952 collection, a single chiasma in the deficient arm was found in 90 percent of the meiocytes while equational separations occurred in 89 percent of the anaphases. For the 1953 collection, the corresponding values were 79 percent for diakinesis and 78 percent for anaphase. Comparisons of the 1952 results with those of 1953 give statistically significant differences: for the diakinesis stages (corrected values), $\chi^2 = 5.1$, df = 1, $P < 0.05$; for the anaphase stages, $\chi^2 = 6.6$, df = 1, $P < 0.02$. As with the heteromorphic A bivalent, the heteromorphic I bivalent gave the same correspondence between frequency of chiasmata and equational separations under two different conditions. Because both sets of examples of the heteromorphic I bivalent were taken from original X_1 subdivisions, environmental factors alone are to be considered responsible for the observed differences.

Desynapsis in the heteromorphic I material of 1953

The meiocytes from the 1952 collection showed regular associations of the normal bivalents. The heteromorphic homologues appeared as univalents in only a small percentage of cases and no laggards were observed at anaphase. In the 1953 material, the heteromorphic homologues, as well as the normal homologues, frequently appeared as univalents at diakinesis (fig. 7) and later stages. The failures in bivalent formation in the 1953 material should be attributed to environmental factors because the collection was obtained from a subdivision of the original X_1 plant which had previously shown normal meiosis. The presence of univalents at diakinesis and metaphase seemed to be the result of desynapsis rather than asynapsis. Examination of pachynema, which is very clear in this material, showed little or no failure in pairing, and, at diakinesis, many of the homologous univalents were lying close together and oriented in a parallel fashion. Both of the heteromorphic homologues frequently lagged at first anaphase and also showed precocious division in a small percentage of meiocytes. Table 3 summarizes the observations of univalents from

FIGURE 1–12.—Diakinesis, metaphase, and anaphase I of heteromorphic bivalents, various enlargements from ca. 500× to ca. 2350×. Figure 1, 2. Heteromorphic A bivalent at metaphase I with no chiasma in deficient arm. Figure 3–5. Heteromorphic A bivalent at metaphase I with one chiasma in deficient arm. Figure 6. Heteromorphic A homologues (arrows) separating reductionally at anaphase I. Figure 7, 8. Heteromorphic A homologues separating equationally at anaphase I, the two dyads from opposite poles of one cell. Figure 9. Heteromorphic I homologues (arrows) unpaired at late diakinesis in material showing desynapsis; note the fairly close approximation of the other two pairs of univalents (connected by dotted lines). Figure 10. Heteromorphic I bivalent at diakinesis with chiasma in deficient arm. Figure 11. Heteromorphic I homologues (arrows) separating reductionally at anaphase I. Figure 12. Heteromorphic I homologues (arrows) separating equationally at anaphase I.

TABLE 3

Univalents at diakinesis in the heteromorphic chromosome I clone, 1953 collection

Configurations of		No. of meiocytes
Normal homologues	Heteromorphic I	
11 II	1 II	185*
	2 I	46
10 II, 2 I	1 II	75
	2 I	12
9 II, 4 I	1 II	20
	2 I	5
8 II, 6 I	1 II	2
	2 I	0

Normal homologues:	Heteromorphic homologues:	
	as bivalent	as univalents
no univalents	185	46
2–6 univalents	97	17

$\chi^2 = 0.96$ df = 1 $P > 0.30$

* In 9 cases, the two homologues were associated by the short arm only; i.e., no chiasma in the heteromorphic arm.

the same two slides which provided the data on chiasmata in table 2b. It is of interest to note that there is no statistically significant difference in frequency of heteromorphic univalents on comparison of the meiocytes which otherwise lack univalents with those which have 2 to 6 univalents. There is thus no evidence here for the sort of competition for chiasmata described by MATHER and LAM (1935) and MATHER (1936). However, among the 345 cells examined, the heteromorphic pair appeared as univalents in 63 while all other homologues gave only 121 pairs of univalents. A negative correlation of chiasma frequencies may, therefore, be masked in this instance by the relatively much greater probability of univalent formation by the heteromorphic rather than the normal pairs.

A PARACENTRIC INVERSION

An X_1 plant showing approximately 50 percent pollen abortion proved on further examination to be heterozygous for a long paracentric inversion. A majority of meiocytes at anaphase I or II had acentric fragments and bridges or loops each of uniform size (table 4c, fig. 24–27). Observations at anaphase II, when the short arms can be easily recognized, enabled an identification of chromosome H as the altered member. Because the inversion chromosome can not be recognized as such during mitosis, it has not, however, been possible to discriminate with complete certainty between chromosome H and the very similar chromosome I.

In *L. formosanum* pachynema is unfavorable for the identification of inversions because of the numerous intertwinings of the long threads. At anaphase I, a comparison of the length of the fragment with that of the loop and noncrossover chromatids

TABLE 4

Chiasmata and crossing over in two X_2 plants heterozygous for a paracentric inversion

*a. Chiasmata within the inverted region at diakinesis; Plant No. 221-11, collected August 30, 1954, Plant No. 221-19, collected September 3, 1954; three slides each from one anther**

	Configuration type †	Plant No. 221-11		Plant No. 221-19	
	Two chiasmata				
1	Symmetric	(7, 9, 9)	25	(8, 9, 8)	25
2	Asymmetric	(14, 6, 16)	36	(16, 12, 16)	44
	Sub-total		61		69
	One chiasma				
	Symmetric				
3	Open	(4, 3, 6)	13	(7, 6, 5)	18
4	Not distinguishable	(6, 5, 8)	19	(6, 7, 3)	16
	Asymmetric				
5	B-type	(7, 3, 8)	18	(4, 3, 4)	11
	$-type				
6	Open	(1, 2, 4)	7	(2, 2, 2)	6
7	Not distinguishable	(2, 3, 5)	10	(1, 1, 2)	4
	Sub-total		67		55
8	*No chiasma*	(7, 3, 7)	17	(5, 1, 3)	9
	Total		145		133
	Non-analyzable	(3, 2, 4)	9	(4, 2, 2)	8

b. Chiasmata exterior to the inverted region at diakinesis; collections and slides as in part a, above

Inversion chiasmata	Plant No. 221-11	Plant No. 221-19	Combined totals
With chiasma(ta) in inverted region			
1 proximal, 1 distal	127	124	
1 proximal	1	0	
Totals	128	124	(252)
With no chiasma in inverted region			
1 proximal, 1 distal	9	7	
2 proximal, 1 distal	0	1	
1 proximal, 2 distal	2	0	
1, proximal or distal	6	1	
Totals	17	9	(26)

* Frequencies for the individual slides are listed in order in parenthesis.

† Types of bivalents are numbered for convenience of reference.

TABLE 4--*Continued*

c. Configurations at anaphase I, collection dates as cited in part a, above, one slide each from a different anther of the same flowers used for diakinesis

Number of fragments	Plant No. 221-11	Plant No. 221-19
2 fragments		
double bridge	11	16
two loops	9	13
Sub-total	20	29
1 fragment		
bridge	39	65
loop	41	58
Sub-total	80	123
0 fragment	21	45
Total	121	197

d. Statistical comparison of frequencies of chiasma formation within inverted region, and crossover products at anaphase I

Plant No. 221-11					Plant No. 221-19				
Frequencies of observed configurations at diakinesis		Expected crossover products at anaphase I			Frequencies of observed configurations at diakinesis		Expected crossover products at anaphase I		
		0 frag.	1 f.	2 f.			0 frag.	1 f.	2 f.
2 chiasmata	61	15.25	30.50	15.25	2 chiasmata	69	17.25	34.5	17.25
1 chiasma	67		67		1 chiasma	55		55	
0 chiasma	17	17			0 chiasma	9	9		
Expected totals		32.25	97.50	15.25	Expected totals		26.25	89.5	17.25
Observed frequencies at anaphase I		21	80	20	Observed frequencies at anaphase I		45	123	29
$\chi^2 = 2.36$ df = 2, P > 0.30					$\chi^2 = 0.81$ df = 2, P > 0.50				

e. Statistical comparison of frequencies of chiasma-formation proximal to the inverted region and types of separations at anaphase I

Plant No. 221-11				Plant No. 221-19			
Frequencies of observed configurations at diakinesis (with 1 or 2 chiasmata in the inverted region)		Expected separation products		Frequencies of observed configurations at diakinesis (with 1 or 2 chiasmata in the inverted region)		Expected separation products	
		Bridge	Loop			Bridge	Loop
1 proximal chiasma	128	64	64	1 proximal chiasma	124	62	62
0 proximal chiasma	0	0	0	0 proximal chiasma	0	0	0
Expected totals		64	64	Expected totals		62	62
Observed frequencies at anaphase I		50	50	Observed frequencies at anaphase I		81	71
$\chi^2 = 0.00$ df = 1, P = 1.00				$\chi^2 = 0.31$ df = 1, P > 0.50			

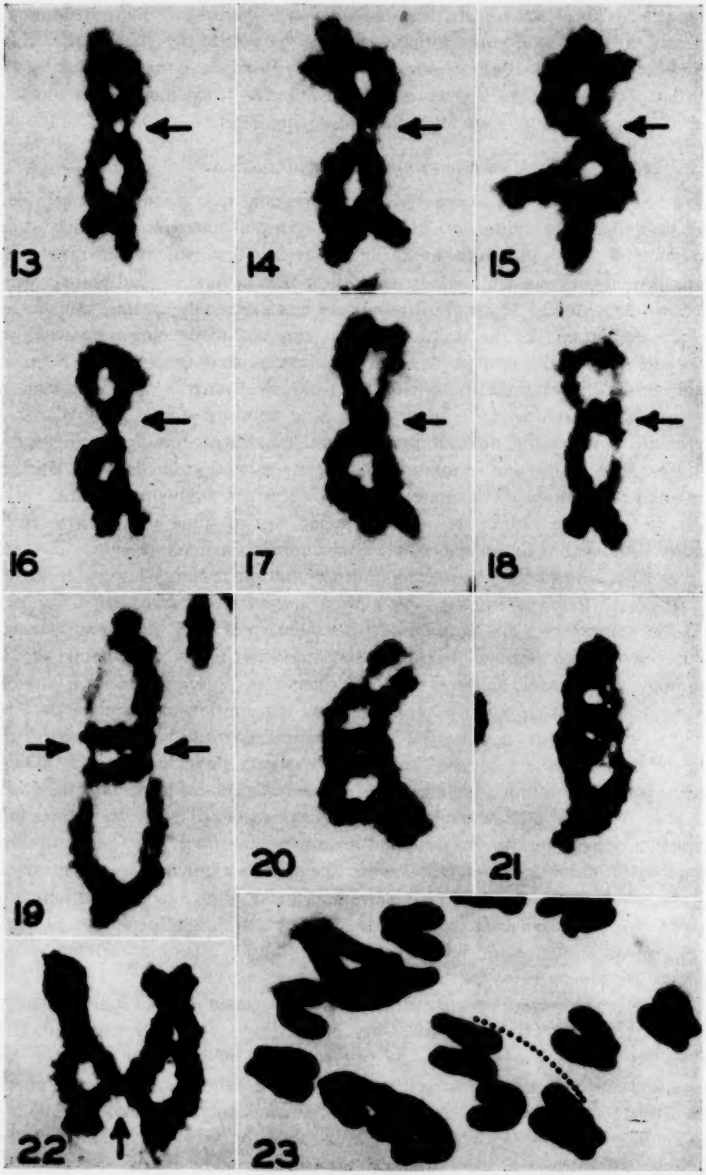
showed the center of the inverted region to be slightly closer to the centromere than to the end of the chromosome. At diakinesis, bivalents with two chiasmata within the inverted segment enabled an estimate of the length of the inversion as at least one third that of the arm. On the basis of these estimates, it was possible to construct a model of the probable pachytene configuration (fig. 28a).

Inversion configurations at diakinesis

According to the chiasmatype hypothesis crossing over within a long inversion should lead to the formation of characteristic types of inversion bivalents at diakinesis. Some of these configurations, if found, would offer evidence in support of the chiasmatype hypothesis while others could be explained just as readily in accordance with a two-plane model. The appearance of the expected configurations will, of course, be determined in part by the morphology and behavior of the chromosomes in which they occur; they will, therefore, be described here as we have observed them in lily chromosomes. The configurations have been observed only in two X_1 plants, each heterozygous for a paracentric inversion, and in only one of the 12 bivalents; they occurred infrequently for the first inversion which had little double crossing over and was not studied further and commonly for the inversion described above. The results of a quantitative study of the latter will be given in the following sections.

A single crossover within the inverted region will produce one acentric and one dicentric chromatid. The single chiasma resulting from such a crossover will therefore be a *reversed chiasma* where two of the four chromatids become changed in direction. Such chiasmata have previously been termed inversion chiasmata by DARLINGTON (1937) and elsewhere; his terminology becomes confusing when two chiasmata occur in the inverted segment. On the assumption that diplotene opening out occurs at random, the reversed chiasma will be either *open* or *interlocked* in a one-to-one ratio (fig. 29). Because only two, instead of four chromatids are continuous through the region of the chiasma, the open reversed chiasma would be expected to form a region of mechanical weakness. The open chiasmata have been readily identified according to the criterion that only two chromatids connect the two parts of the bivalent at this point, and, in contrast to ordinary chiasmata, many were considerably stretched on smearing. In the first few examples observed, the "yoke bivalents" reported by BROWN and ZOHARY (1953), the two continuous chromatids were so badly stretched that they were no longer identifiable as such; configurations of this type were not analyzed with certainty until after unstretched examples and others with intermediate degrees of distortion had been found. Examples of open, reversed chiasmata are shown in fig. 13, 14, 16, 17.

If a single crossover occurs near either end of the inverted region, an asymmetric bivalent (fig. 14–18, 28b) will result which has either a B- or $-shape depending on whether the two long segments lie on the same or opposite sides of the bivalent. Presumably the difference between the B- and the $-bivalents is determined by chance during the smearing process. The external chiasmata usually found in the inversion bivalent have without doubt been partially responsible for the internal stretching which gives a very striking appearance to many of the asymmetric bivalents. Several hundred clear examples of asymmetric bivalents have been



FIGS. 13–23

observed in preparations from different collections. In general, the open reversed chiasmata have been much more readily identified in the S- than in the B-bivalents.

If double crossing over occurs within the inverted region, the part of the inversion loop between the points of crossing over will be arrested between the two resultant chiasmata in the diakinetic bivalent (fig. 19–21, 28c). Both of the chiasmata will be measured whether from 2-, 3-, or 4-strand crossing over. If the two points of crossing over are equidistant from the center of the inversion, the bivalent will appear symmetrical. If one is farther removed from the center than the other, the bivalent will be asymmetric, and the asymmetry will always be of the B-type because both long segments will be attached to the same side of the arrested loop segment. Numerous examples of double crossing over within the inverted region have been identified; because of the compact nature of these bivalents, open reversed chiasmata have not been readily identified although frequently one of the two chiasmata will show the mechanical weakness expected with the open form.

Scoring of inversion configurations

Material for the two quantitative comparisons each came from a single flower one anther of which provided material for one or two slides of anaphase I, another, for three slides of diakinesis. For both stages, the cells were examined first under low power (150×). The anaphase I examinations were restricted to isolated cells at mid-anaphase and all were analyzable. For diakinesis, only those cells which showed twelve clearly separated bivalents were chosen for further study. This stringent selection was necessary to assure favorable material for the quantitative classification of the inversion configurations; they are only rarely recognizable under low power so that little or no bias was to be expected from this method of selection.

Of the eight types of diakinetic configurations listed in table 4a, all but two, types 4 and 8, could be readily recognized under oil immersion (1350×). With types 4 and 8, the inversion bivalent had to be distinguished from one other bivalent very similar to it in size. If both of these bivalents either had or lacked a chiasma in the proper region, no further discrimination was necessary. In a few instances, only one of the two bivalents had a chiasma in the appropriate region and in these cases the identification of the inversion bivalent was based on the fact that its external chiasmata are usually sharply localized with the distal one being about twice as far as the

FIGURE 13–21.—Inversion bivalents at diakinesis (ca. 2100×). Figure 13. Symmetric bivalent with open reversed chiasma (arrow). Figure 14. Asymmetric, $-shaped bivalent with open reversed chiasma (arrow). Figure 15. Asymmetric, $-shaped bivalent with reversed chiasma (arrow) in which the chromatid arrangement is not distinguishable. Figure 16. Asymmetric, B-shaped bivalent with open reversed chiasma (arrow) in which the two continuous chromatids are superimposed. Figure 17. Asymmetric, B-shaped bivalent with open reversed chiasma (arrow). Figure 18. Asymmetric, B-shaped bivalent with reversed chiasma (arrow) in which the chromatid arrangement is not distinguishable and the two longer segments are overlapping. Figure 19. Asymmetric bivalent with two chiasmata in the inverted region (arrows); the axis of the arrested loop segment is at right angles to the bivalent; the chiasma indicated by the left arrow is apparently an open reversed chiasma. Figure 20, 21. Asymmetric bivalents with two chiasmata in the inverted region. Figure 22. Quadrivalent from tetraploid material demonstrating interstitial chiasma (arrow); (ca. 2100×). Figure 23. Precocious separation of centromeres in anaphase I (ca. 1700×); dotted lines connect homologous dyads; the two daughter centromeres of the upper dyad are widely separated.

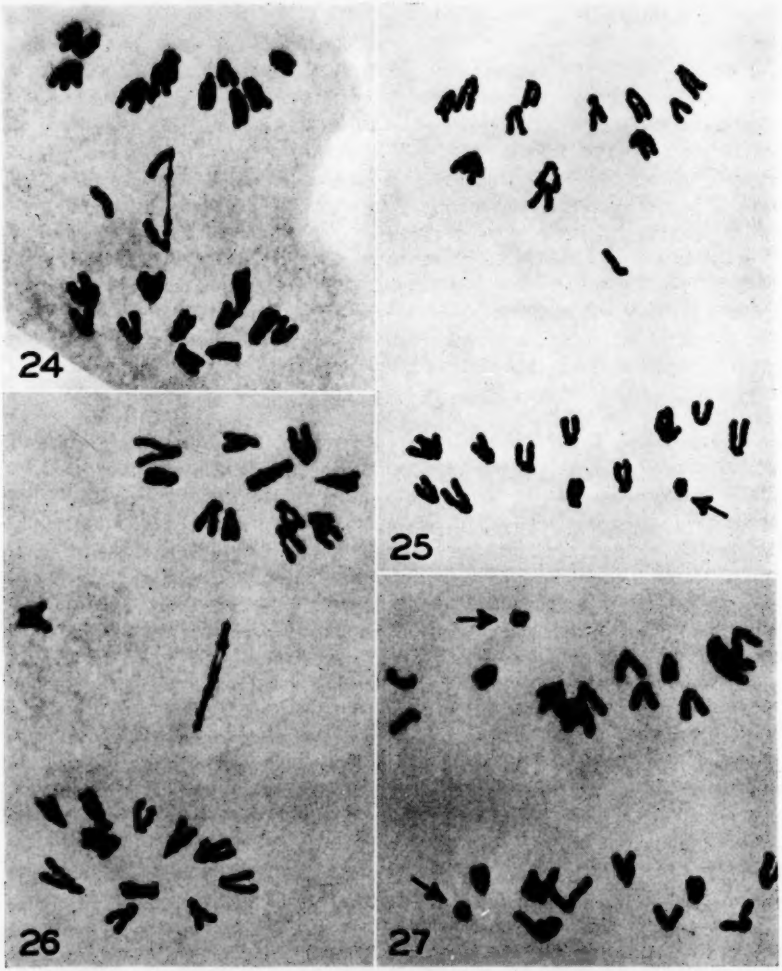


FIGURE 24–27.—Products of crossing over in the inverted region at anaphase I (ca. 650×). Note uniform size of fragments and loops. Figure 24. Single chromatid bridge and single fragment. Figure 25. Single loop chromatid (arrow) and single fragment. Figure 26. Double chromatid bridge and two fragments adhering to each other. Figure 27. Two loop chromatids (arrows) and two fragments.

proximal from the adjacent chromosome end (fig. 13–21). It seems likely that not more than two or three of the bivalents tallied in table 4a could have been misclassified, and these would be, and with apparently equal probability in each case, either type 4 or type 8 mistaken for the other.

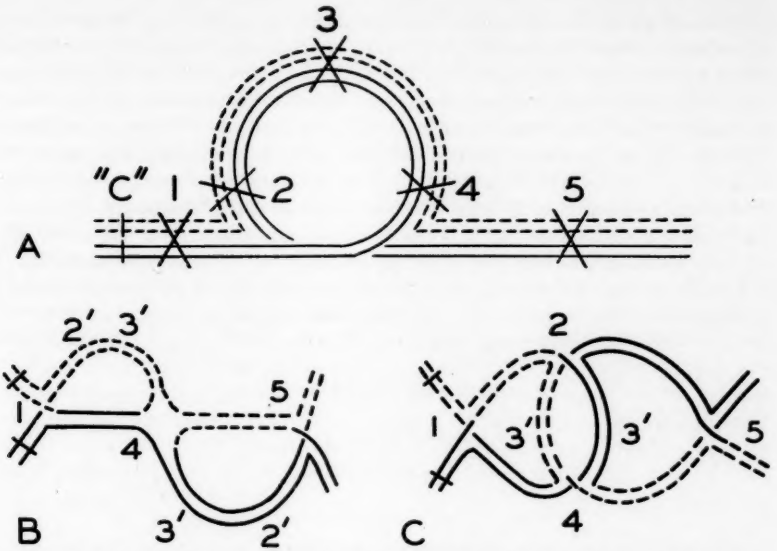


FIGURE 28.—A. Model of probable pachytene configuration of the inversion bivalent. "C" marks centromere position. The numbers, 1–5, refer to regions in which crossing over may occur. Regions 1, 5 are exterior: 1, proximal, 5, distal. Regions 2–4 are within the inverted segment. Single exchanges at 3 produce symmetric bivalents, at 2 or 4, asymmetric. Double exchanges at 2 and 4 produce symmetric bivalents, at 2 and 3 or 3 and 4, asymmetric.

B. Diagram of the chromatid relationships in a diakinetic bivalent expected according to the partial chiasmatype hypothesis following crossing over in regions 1, 4, and 5. Regions in which crossing over did not occur are indicated by primes. This diagram of an asymmetric $-shaped bivalent shows only one possibility; the same general relationships would exist in the B-type of asymmetry, and the reversed chiasma (4) could be either open, as shown, or interlocked (see fig. 29).

C. Diagram of the chromatid relationships in a diakinetic bivalent expected according to the partial chiasmatype hypothesis following crossing over in regions 1, 2, 4, and 5. Following double crossing over within the inverted segment the portion between the two exchanges, between regions 2 and 4, becomes arrested and will appear to be held at right angles to the main axis of the bivalent.

The unanalyzable cases at diakinesis were those in which the orientation of the bivalent prevented analysis of the chiasmata although the inversion bivalent was sometimes recognizable as such. Because of its more compact nature, the inversion bivalent with two chiasmata might be expected to contribute disproportionately to the unanalyzable class, and, as will be shown later, this class seems to be deficient.

Quantitative comparisons, internal chiasmata

Table 4a lists the various types of configurations for chiasmata in the inverted segment and the frequencies in which they were observed at mid-diakinesis. The open reversed chiasma (fig. 29) may be readily identified at this stage when a single chiasma only is present and the asymmetry is not of the B-type. However, some clear examples of open chiasmata have been seen also in B-configurations. The interlocked

reversed chiasmata have not been critically demonstrated although in some cases where the configurations are stretched, and overlapping eliminated, interlocking seems the most likely interpretation. If there is a random relationship among the chromatids when the homologues open out, then a 1:1 ratio between the open and interlocked types of reversed chiasmata is to be expected. This ratio is closely approximated by the proportion of those identifiable as open (table 4a, types 3, 6) to those not thus identifiable (types 4, 7). Considering both plants together, there is thus a total of 44 open to 49 not distinguishable or an excellent agreement with the expected 1:1 ratio. Although this quantitative comparison does not prove the existence of the interlocked type, it does give indirect evidence for its occurrence.

A single chiasma laterally placed in the inverted region will yield an asymmetric configuration (table 4a, types 5–7); a centrally placed chiasma will yield a symmetric bivalent (types 3, 4). With two chiasmata, the configuration will be symmetric if both

FIGURE 29.—Diagrams showing the chromatid relationships in open and interlocked reversed chiasmata (see text for further explanations) and of an ordinary chiasma for comparison.

are lateral (type 1), and asymmetric if one is lateral, the other central (type 2). The ease with which the symmetric types could be distinguished from the asymmetric testifies again to the pronounced localization of chiasmata in this material. A lateral chiasma may occur in either of two positions while a central chiasma may occur in only one (fig. 28a). Our material possessed no sort of cytological marker, such as a large knob, which would aid in distinguishing the two types of lateral chiasmata; however, an assumption of equal or other relative frequency for the two types is unnecessary for the considerations to follow. It becomes apparent from inspection of the data that there is a much higher frequency of lateral chiasmata when two chiasmata, rather than one, are present in the inverted region. For the following estimate, the frequencies for the two plants summarized in table 4a will be combined. For the single chiasmata, there are 66 central (types 3, 4) to 56 lateral (types 5–7). For the types with two chiasmata, there are 50 with two lateral (type 1), and 80 with

one lateral, one central (type 2) or a total of 80 central to 180 lateral chiasmata. The marked difference in the ratios, 66:56- and 80:180-central:lateral, respectively, may be a reflection of chromosomal interference but may also be partially the result of pairing complications in the region near the ends of the inverted segment. This difference may also be considered in light of the equal expectation, one central to two lateral chiasmata, for both one and two chiasmata on the assumption of equal probability for chiasma formation in each of the three regions.

On the assumption of random relationship among the four chromatids involved in two crossovers, it is possible to calculate from the diakinesis data the frequencies of the expected crossover types and to compare these with those observed at the subsequent anaphase. Table 4d shows the calculations and the comparisons; because the expected values were derived from a sample, a 2 × 3 table was used in making the chi-square test. In general, there is good agreement between the observed and expected frequencies; the major discrepancy is to be found in the larger expectation for the 0-fragment and the smaller expectation for the 2-fragment crossover types than were actually observed. This discrepancy, if not merely a sampling error, may in part be attributed to the somewhat greater difficulty of identifying bivalents with two chiasmata (types 1, 2) because of their more compact nature.

Quantitative comparisons, external chiasmata

As may be seen from table 4b, all but one of the 252 bivalents with chiasmata in the inverted region also have two external chiasmata. Among the 26 bivalents without chiasmata in the inversion, 7 had only one external chiasma. This observation conforms to cytological demonstrations of asynapsis and non-homologous associations in chromosomes with various sorts of rearrangements (McClintock 1933).

On the assumption of a random relationship among the four chromatids involved in the various exchanges, anaphase I bridges, whether single or double, will be changed into loops in half the cases in which a single proximal chiasma is formed. The almost constant occurrence of a proximal chiasma leads to the expectancy of a 1:1 ratio between loops and bridges at anaphase I. That this expectation is borne out very closely is demonstrated by the anaphase observations reported in table 4c and the statistical comparisons of table 4e; because the expected values were themselves derived from observed samples, 2 × 2 tables were used for the calculation of chi-square. As is the case with the chiasmata in the terminal deficiencies, the evidence from the proximal chiasmata must be considered in relation to the possibility of equational separation of the centromere.

It would seem reasonable to expect the two loop chromatids resulting from certain triple crossovers (2 within, 1 proximal to the inverted region) to be not infrequently interlocked and to appear so at anaphase I. Upcott (1937) explained the occurrence of an example of interlocking of bridges at anaphase II by the prior interlocking of two loops at anaphase I but did not directly demonstrate the latter. Among the anaphase I cells tallied in table 4c we found no cases of interlocking of two loops and only one example during a careful search among a considerable number of other cells with two fragments. Although the expected percentage of interlocking would depend on the mode of crossing over envisaged, all those we have considered lead to a

higher expectation than that actually found. This interesting problem can not be further discussed in this report but its study is being continued.

For the work with the inversion, two different X_2 plants were used, and these presumably would differ genetically. The samples, however, were collected at about the same time under good growing conditions. If the frequencies at diakinesis of the 0, 1, and 2-chiasma types are compared, there is no significant difference between the two plants, with $\chi^2 = 3.58$, df = 2, $P > 0.10$.

OTHER OBSERVATIONS

Two other observations made during the course of this study are of interest in connection with the relationship of chiasmata to crossing over, and will be briefly described.

The first of these concerns the precocious equational division of the centromere. In some of the material collected from an inversion heterozygote during the poor climatic conditions early in the growing season, clumps of cells were found, among the normal meiocytes, in which some or all of the centromeres had separated equationally during anaphase I (fig. 23). In all such precocious separations, both sister centromeres retained the orientation toward the original pole; among the scores of cells observed, no examples were found in which the two sister centromeres had become oriented toward opposite poles. The significance of this observation will be considered further in the discussion.

In tetraploid material, it was possible to find good examples of quadrivalents with interstitial chiasmata. Such chiasmata have been considered by DARLINGTON (1930) and DARLINGTON and MATHER (1932) to provide evidence in favor of the chiasmatype hypothesis. A photograph is offered of one example in which the change of chromatid partners is evident at the interstitial chiasma (fig. 22). This observation thus provides another confirmatory piece of evidence from the species under consideration.

DISCUSSION

Heteromorphic homologues

In only a few cases have heteromorphic bivalents been used for analyzing the quantitative relationship between chiasmata at diakinesis and type of separation at anaphase. Of the studies on this subject already cited in the introductory section, only that of HAGA (1944) has given results at variance from those expected on the basis of the chiasmatype hypothesis. In Paris HAGA found a significantly higher proportion of equational separations at anaphase I than of chiasmata in the deficient arm at metaphase I. However, the centric regions of the Paris chromosomes remain paired until anaphase separation begins so that chiasmata immediately adjacent to the centric region would be difficult to identify. Thus, the excess of equational separations reported by HAGA is not unexpected in view of the complication imposed by such special centric association.

In Lilium, the present analysis of the heteromorphic homologues and of the proximal chiasmata in the inversion bivalent was simplified by the fact that not more than one chiasma was present. In use of this information as evidence in support of the partial chiasmatype hypothesis one also should consider, however, the possibility

that the centromere may separate equationally as well as reductionally. There are three reasons for believing that equational centric separations occur rarely, if at all, in Lilium. In the first place, an equational separation would yield two chiasmata, one on either side of the centromere. To explain why such paired chiasmata do not occur the further assumption must be made that a chiasma in the short arm may slip off, that is, terminalize and then disappear completely. We have, however, no evidence that chiasmata terminalize in Lilium; our counts at early diakinesis and metaphase agree well with those of MATHER (1935) in showing little or no terminalization between these stages. Secondly, the frequency of chiasma formation in those regions where centromere separations need to be considered is of the same general order as that for the inversion region where none of the chiasmata could have been formed by equational centric separations. Lastly, Lilium has a very typical meiotic sequence and there seems, therefore, no reason for not extending to this genus a generalization of the many observations, from diverse organisms, of the reductional separation of the centromeres at the first anaphase: Drosophila, (BRIDGES and ANDERSON 1925); Zea, (RHOADES 1931; MCCLINTOCK 1933); Neurospora, (LINDEGREN 1933); Lycopersicon, (BARTON 1951); Triticum, (HUSKINS and SPIER 1934); the mole, Talpa, (KOLLER 1936a); several marsupials, (KOLLER 1936b); several Orthopterans (CAROTHERS 1913). For these three reasons the information obtained from the heteromorphic homologues and from the proximal chiasmata of the inversion bivalents would seem to be acceptable for demonstration of the relationship of crossing over to chiasma formation.

A parallelism between the influence of temperature on chiasma formation and crossing over has been noted by WHITE (1934) in comparing his cytological observations on three species of Acridiidae with the older genetic data gathered by PLOUGH (1917) from Drosophila. Although the specific environmental factors responsible for differences have not been identified in the present study, a similar parallelism between crossing over and chiasmata is apparent on comparing the frequencies for the two collections of each of the two deficiencies.

Inversion configurations

Evidence in support of the partial chiasmatype hypothesis is provided by the analysis of the inversion heterozygotes. The open reversed chiasmata and the asymmetric configurations seen at diakinesis are inexplicable on a two-plane model unless the unlikely assumptions are made of pairing and chiasma formation in non-homologous regions. MATSUURA (1950) has very clearly outlined the types of configurations which are to be expected on the basis of a two-plane model and none of these have been observed at all. Furthermore, the frequency of chiasma formation at diakinesis is not at variance with the frequency of crossover types which appear at anaphase I. The regularity of the occurrence of the various inversion configurations at diakinesis also supports the partial chiasmatype hypothesis. From this point of view the extreme rarity of critical diakinetic configurations in earlier examinations of inversion bivalents is certainly unfortunate. The combined efforts of SMITH (1935), RICHARDSON (1936), DARLINGTON (1936), DARK (1936) and UPCOTT (1937) resulted only in the observation of a few examples. UPCOTT (p. 349) found two asymmetric

bivalents in Tulipa. A single case of an open reversed, or "inversion" chiasma was reported by DARLINGTON in Chorthippus. Five others were seen by SMITH in Trillium and DARK reported two more for Paeonia. It seems most likely that these workers were dealing with small inversions in which chiasma formation was rather rare. The small size of an inverted region would be sufficient explanation for the failure of formation of detectably asymmetric bivalents. The smearing techniques employed earlier did not result in much flattening of the chromosomes and therefore would make the detection of open reversed chiasmata rather difficult.

Conflicting evidence and hypotheses

In a dicentric chromosome in wheat a chiasma formed between the primary and secondary centromeres would be expected to produce a double bridge at anaphase. In their study of this chromosome STEINITZ-SEARS and SEARS (1953) found only 9.1% bridges in striking contrast to 28.0% chiasmata between the two centromeres; they concluded that these data could not be satisfactorily reconciled with the partial chiasmatype hypothesis. However, other explanations were not entirely excluded and among these was that of premature separation of secondary sister centromeres. DARLINGTON (1939) has summarized and interpreted various observations of a transverse stretching of the centric region under stress at anaphase I. It seems plausible that the small secondary centromeres of the wheat dicentric might simply split rather than stretch transversely when under stress. The observations reported above on the precocious equational separation of sister centromeres in *L. formosanum* demonstrate that such separations may not be detectably associated with any change in activity or orientation. Thus the failure of the wheat secondary centromere to show activity at anaphase I cannot safely be used as a criterion for judging the ease with which the sister secondary centromeres might be pulled apart.

COOPER (1949) has successfully demonstrated that chiasmata or chiasmata-like configurations may be formed in meiosis in male *Drosophila melanogaster*, yet few or no crossovers appear. Likewise, the frequency of chiasmata in paired somatic chromosomes (KAUFMANN 1934; COOPER 1949) is seemingly much higher than the amount of somatic crossing over, although the two processes have not been studied in the same tissue. It thus seems apparent that a chiasma cannot safely be assumed to represent a prior crossing over in all organisms. One of the future tasks of comparative cytology will be to determine when such an assumption may be made with reasonable assurance of its validity. In addition, the presence of such unusual features as somatic pairing in the fruit fly may help to provide valuable clues as to the nature of the chiasmata under consideration.

SAX (1930, 1932) suggested the idea that crossing over occurs through breakage and union at chiasmata originally formed by alternate equational and reductional openings at diplotene. Although later abandoned by SAX (1936), it should be noted that his idea becomes almost identical with the partial chiasmatype hypothesis if the equational separations are assumed to extend over only very small regions and one of each pair of chiasmata then always becomes resolved in early diplotene. If SAX's scheme thus modified were applied in the present instance, two chiasmata would have originally been present in the very short deficient arm of the heteromorphic A chromosome. As pointed out by DARLINGTON and MATHER (1932) the regular

occurrence of pairs of chiasmata so close together would be unlikely if judged by the distribution of chiasmata apparent at diakinesis; however, if crossing over regularly follows the formation of pairs of closely juxtaposed chiasmata, these pairs would not be distributed in the pattern perceptible at later stages. SAX's hypothesis as so modified may thus receive confirmation or disproof only when the mechanism of crossing over becomes understood.

In recent years MATSUURA (1937, 1938, 1950) and HAGA (1944, 1953) have been the outstanding defenders of the two-plane hypothesis. In 1950 MATSUURA very clearly described the diplotene or diakinetic configurations to be expected in inversion bivalents according to his neo-two-plane hypothesis and according to the partial chiasmatype hypothesis. He recognized that asymmetric inversion configurations (with single chiasmata) are to be expected only on the basis of the chiasmatype hypothesis. As previously mentioned, the types of configurations to be expected on the basis of the neo-two-plane hypothesis (MATSUURA 1950, p. 53) have not been observed at all in our material although they would have been expected to provide some very striking configurations. Thus, our observations on Lilium conform to MATSUURA's requirements for the partial chiasmatype hypothesis rather than to those for the neo-two-plane hypothesis.

Time of crossing over

In 1932 CREIGHTON and MCCLINTOCK presented a demonstration of four strand crossing over in which the crossover products were identifiable at diakinesis. Much of the work summarized in the introduction would also point to stages prior to diakinesis or mid-diplotene as the time of occurrence. In the Lilium material reversed chiasmata have been clearly observed at late diplotene or early diakinesis. It thus seems most probable that meiotic crossing over usually occurs sometime between the onset of pairing and the completion of the diplotene opening of the bivalents.

SUMMARY

Two terminal deficiencies and a paracentric inversion, resulting from X-ray treatment of pollen, were used for a study of the relationship of chiasmata and crossing over in *Lilium formosanum*.

In plants heterozygous for either terminal deficiency, there was a close correspondence between the frequency of a single chiasma in the deficient arm and of equational separations at anaphase. This correspondence proved true for two different environmental circumstances for each deficiency. In plants heterozygous for the inversion, the regular occurrence of a chiasma between the centromere and the inverted region led to an expectation of a 1:1 ratio for dicentric bridge and loop chromatids at anaphase I; this expectation was closely borne out by the anaphase observations. Reasons are given in the discussion for believing these observations are not to be explained by equational centric separations.

Chiasmata in the inversion region are frequently directly recognizable because of the formation of "reversed" chiasmata, asymmetric configurations, and the characteristic configuration when two chiasmata are present. The occurrence of reversed chiasmata and of asymmetry afford evidence in support of the partial chiasmatype hypothesis. On the assumption of a random relationship among the chromosomes

involved, the frequency of crossing over observable at anaphase I was not at variance from that expected on the basis of chiasma formation at diakinesis.

The discussion includes a brief consideration of earlier work with heteromorphic and inversion configurations, of observations at variance to the partial chiasmatype hypothesis, and of the time of crossing over.

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CHIASMATA IN A PERICENTRIC INVERSION IN ZEA MAYS

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INVERSIONS can provide critical material for testing the validity of the partial chiasmatype hypothesis. According to this hypothesis crossing over within an inversion should lead to the formation of several characteristic bivalent configurations in diplotene and diakinesis. As pointed out by DARLINGTON (1937) and recently by BROWN and ZOHARY (1955), some of these configurations, namely inversion asymmetry and the open reversed inversion chiasma, could offer, if found, critical indication that crossing over actually leads to formation of a chiasma.

Such inversion configurations have been observed by BROWN and ZOHARY (1955) to occur regularly in microsporocytes of *Lilium formosanum* heterozygous for a long paracentric inversion. For confirmatory evidence it was felt desirable to look for such configurations in other organisms. The present paper reports the results of such a search in a case of a long pericentric inversion in chromosome 1 of maize.

MATERIALS AND METHODS

Several seed of *Zea mays*, obtained from selfing a plant heterozygous for a pericentric inversion in chromosome 1 (points of breakage: 1S.3–1L.5), were grown at Berkeley in the summer of 1954. A plant heterozygous for this inversion was selected from this group after identification of the inversion loop at pachynema. Spikelets from this plant were fixed in 3:1 alcohol-acetic acid for 24 hours. Anthers were smeared in aceto-carmine and temporary slides were used for examination and photography, one to four days after their preparation.

Throughout this study, microsporocytes have been first observed under low dry magnification. Only these cells in which all the ten bivalents could be seen to be well separated from one another were chosen for further observation.

OBSERVATIONS

Microsporocytes in mid-diakinesis were found to be the most suitable material for the present study. In addition, observations have been made also in early diplotene. Late diplotene and early diakinesis stages could not be utilized, since cells in these stages showed considerable stickiness and clumping of chromosomes together. Metaphase I bivalents were too compact for reliable identification of chiasmata.

Early diplotene

Several inversion bivalents have been observed at this stage in which the pachytene inversion loop (or part of it) was still arrested (fig. 1 & 2). The bivalent photographed in figure 2 can perhaps best be interpreted as having two chiasmata within the inversion. However, the general absence of strong repulsion at this stage in regions

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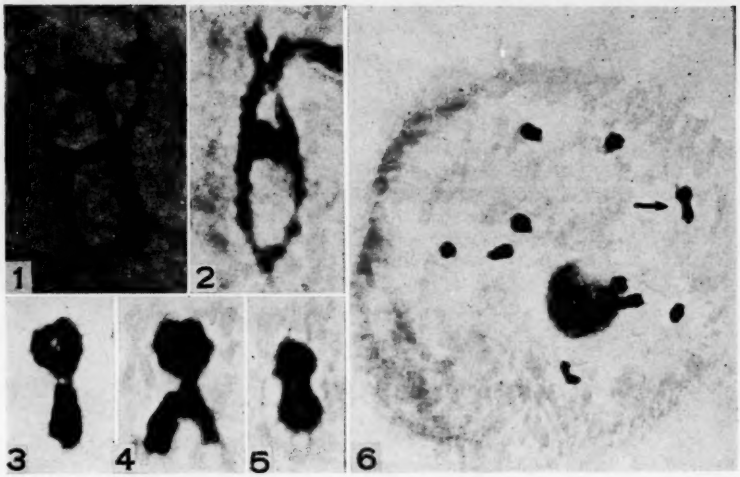

FIGURES 1 and 2.—Early diplotene inversion bivalents, 2250×.

FIGURES 3 and 4.—Mid-diakinesis bivalents with open reversed chiasma and additional terminal chiasma. Bivalent in figure 4 shows apparent inversion asymmetry, 1900×.

FIGURE 5.—Mid-diakinesis bivalent with open reversed chiasma and terminal chiasmata in both arms, 1900×.

FIGURE 6.—The whole microsporocyte of the bivalent photographed in figure 3. Arrow points to the inversion bivalent, 510×.

adjacent to the centromere together with the differential heavy staining in this area (fig. 1), made it difficult to discriminate here between true chiasmata and mere adhesions. For this reason observations at this stage could not be utilized critically for the present study.

Mid-diakinesis

Approximately 250–300 well-flattened microsporocytes have been examined at this stage and at least 9 clear cases of an open reversed inversion chiasma could be identified in chromosome 1 bivalents. Four of these bivalents each had one terminal chiasma in addition to the inversion chiasma (fig. 3 & 4), while the remaining five had terminal chiasmata on both arms (fig. 5). Inversion asymmetry could be noticed in two of these nine bivalents (fig. 4). Clear identification of the asymmetry in these two bivalents was easy since the exact place of the inversion chiasma was clearly marked by the two stretched chromatids.

The small size of the mid-diakinetic bivalents made the detection of other expected inversion configurations (for detailed description see BROWN and ZOHARY 1955) difficult or impossible. Inversion asymmetry was suspected to be present in several other chromosome 1 bivalents which did not show an open reversed inversion chiasma; but the compactness of the chromosomes at this stage made any such identification inconclusive. For the same reasons other types of inversion configura-

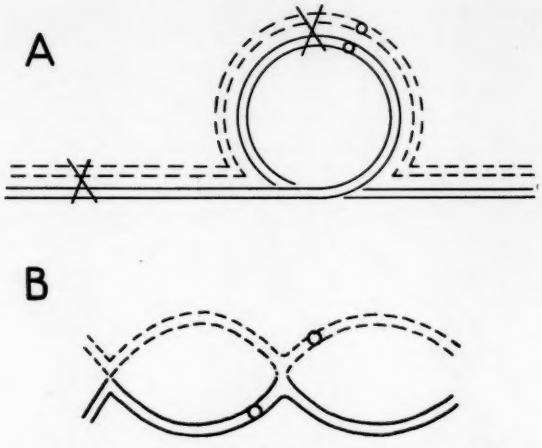

FIGURE 7.—A. Model of pachytene configuration of the inversion bivalent. A single crossing over is assumed to occur within the inversion loop and another one outside it, in the long arm. B. Diagram of one type of expected diakinesis configurations following crossing over in these regions, according to the partial chiasmatype hypothesis. The chiasma between the centromeres is an open reversed inversion chiasma.

tions (such as bivalents with 2 chiasmata within the inversion), even if present, could not be safely identified.

DISCUSSION

Maize chromosomes are too small and compact to be suitable for thorough study of all types of diakinetic and metaphase I inversion configurations. However, several of the expected critical types of bivalents could nevertheless be identified. Perhaps the most important result of this study is the demonstration of the occurence of an open reversed chiasma in some of the diakinetic chromosome 1 bivalents. This type of chiasma can be interpreted only as a result of a previous crossing over within the inversion. The asymmetry which accompanied some of these chiasmata could again be explained only by assuming previous occurrence of a single crossing over at one end of the inversion segment.

These observations, together with the one to one correspondence between crossing over and chiasma formation reported by BEADLE (1932) in a Zea-Euchlaena hybrids, indicate that in *Zea mays* crossing over leads to the formation of a chiasma. They present, therefore, additional evidence for the general validity of the partial chiasmatype hypothesis.

SUMMARY

Open reversed inversion chiasmata and inversion asymmetry were observed at diakinesis in a *Zea mays* plant heterozygous for a pericentric inversion. These configurations indicate that, in maize, crossing over leads to the formation of a chiasma.

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BEHAVIOR OF NUCLEI AT CONJUGATION IN PARAMECIUM AURELIA. I. EFFECT OF INCOMPLETE CHROMOSOME SETS AND COMPETITION BETWEEN COMPLETE AND INCOMPLETE NUCLEI[1]

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THE sexual processes of conjugation and autogamy in *Paramecium aurelia* involve synchronous division of nuclei, movement of nuclei into specific cytoplasmic regions, and survival of some nuclei and death of others. SONNEBORN (1951, 1955) has pointed out the importance of these phenomena for the problem of interactions between nucleus and cytoplasm and has especially emphasized the role of differentiated cytoplasmic regions. NANNEY (1953) has discussed similar problems in Tetrahymena. The present study describes the behavior of grossly deficient products of meiosis from haploid micronuclei and demonstrates that the genetic constitution of a nucleus can play an important and autonomous role in its behavior.

MATERIAL AND METHODS

Two stocks differing from each other in five independently assorting genes were used to study nuclear behavior, SONNEBORN's stock 90 of variety 1 and a synthetic stock called CD. CD is nearly isogenic with 90 except for the five genes *cl* (clear), *dp* (dumpy), *mt* (the one-type allele of the mating type gene), d^{60} (the stock 60 allele of the gene for the D antigens), and g^{60} (the stock 60 allele of the gene for the G antigens). Stock 90 contains the dominant wild type alleles of *cl*, *dp*, and *mt* and the stock-90 alleles of *d* and *g*. We are indebted to DR. G. H. BEALE for furnishing the stock 60 alleles of *d* and *g* in a stock 90 genetic background.

The genes *cl* and *dp* have been briefly reported by KIMBALL (1953). Clear animals lack the crystals found in the cytoplasm of wild type paramecia, and the character can be scored in individual paramecia even if they grow poorly. Dumpy animals differ from wild type in shape, and the character can only be scored in cultures which have grown at normal rate. The behavior of the *d* and *g* genes has been described by BEALE (1952, 1954). Any single paramecium may be immobilized by antisera against antigens controlled by one of these genes but not the other. In the present work, the D antigens determined by the *d* alleles were usually used and no attempt was made to test for the other gene. Animals heterozygous for d^{60} and d^{90} react with both anti-60D and anti-90D serum while the homozygotes react with only one. In most of the work only two genes of the five, *cl* and *d*, were used to follow nuclear behavior.

Haploid and haploid-diploid clones

Haploid clones were produced by irradiating CD with about 30 kr of X-rays and crossing to unirradiated 90. It will be reported in another paper that heavily irradiated animals frequently fail to contribute a male gamete nucleus to their unirradiated

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mates, and the nuclear apparatus in the latter frequently develops from a single haploid product of meiosis. Such haploids have the markers from stock 90 but none from CD. SONNEBORN *et al.* (1953) have briefly discussed the production of haploids by another technique and have pointed out that diploidization frequently occurs. In our experience, diploidization usually occurs in the early history of the clone if at all.

Two haploid clones, 3b and 19b, furnished the main body of data on haploids. The micronuclei of these clones had chromatin rings which were distinctly smaller than normal, and their haploidy was further confirmed by the death of all their progeny by autogamy. In the same experiment from which 3b was obtained, another clone, 20b, was found which, on microscopic examination, proved to contain one large and two small micronuclei. Occasionally more than two small nuclei were found but the nuclear constitution was, on the whole, quite stable. The breeding behavior of this clone is explicable on the assumption that the large nucleus was diploid and the two small ones were haploid. The clone had the phenotype expected if its macronucleus were derived from stock 90 only, and its breeding behavior shows that the diploid and at least one haploid micronucleus must have come from 90 only. It is unlikely, though not impossible, that the other haploid nucleus was from CD and not 90.

Crossing and determining the phenotype of the exconjugant clones

Pairs between the haploid or the haploid-diploid clones and stock CD animals were isolated. For convenience, they were left overnight and then the two members of the pair were put in separate containers using clear and dumpy to ascertain the cytoplasmic origin. The clones were allowed to multiply for 4 days in a small quantity of culture fluid and then classified for the amount of growth, the presence of crystals, and the serotype. Clones were classified as having crystals if some but not all the individuals contained them. Two categories of growth are distinguished in the paper, G and P. Clones classified as G reached a maximum population in 4 days; clones classified as P either died or failed to reach a maximum population in 4 days. In almost all instances, the two categories were markedly different from each other.

CONJUGATION

Normal

Conjugation in Paramecium has been described many times (e.g., SONNEBORN 1947; BEALE 1954), but a summary of the major nuclear events may be useful for an understanding of the rest of the paper. *P. aurelia* normally contains two micronuclei and a macronucleus. The micronuclei undergo meiosis nearly synchronously in the two conjugants to produce eight products in each. In each conjugant one of these enters the paroral cone, a cytoplasmic projection which presses into the side of the other conjugant. This product undergoes a mitotic division while the other seven products rapidly degenerate and are resorbed. One of the products of this mitosis, the male gamete nucleus, remains in the cone and eventually penetrates into the mate. The other, the female gamete nucleus, moves back into the body of the animal during the division at which it arises and later fuses with the male gamete

nucleus from the mate to form the synkaryon. A whole new nuclear apparatus, macronucleus and micronuclei, is derived from this synkaryon, following separation of the conjugants. The two exconjugant clones, one from each conjugant, thus come to have genetically identical nuclei since the two kinds of nuclei which fuse to form one synkaryon are identical with the two that fuse to form the other.

Two major aberrations of conjugation are relatively well known (e.g. SONNEBORN 1949; BEALE 1954). In cytogamy, the male gamete nucleus of each conjugant fuses with the female gamete nucleus of the same conjugant in a double autogamy. In macronuclear regeneration, the macronucleus of the exconjugant clone develops from a fragment of the old macronucleus instead of from the synkaryon. Thus the phenotype of the cytoplasmic parent is retained regardless of the origin of the synkaryon.

Cytological observations

All major stages in the conjugation of haploids with diploids have been observed cytologically though certain stages have been represented by only a few pairs. A number of pairs involving clone 20b were also examined including several in late meiotic and postmeiotic stages. No consistent differences have been found between haploid and diploid micronuclei in the rate at which they proceed through meiosis and the subsequent stages. The variations which have been found are no greater than are found in conjugation between normal diploids and do not seem to be in one direction. Likewise no differences have been found in the rate of macronuclear breakdown. The positions of the micronuclei in the cell at all stages up to and including the postmeiotic interphase seem to be quite variable both in pairs involving haploids and in pairs between normal diploids. The observations are in agreement with WICHTERMAN'S observations (1953) of extensive movement of the nuclei in living *P. caudatum* as a result of cytoplasmic cyclosis.

The only abnormality which was seen in 3b and 20b but not in normal diploids was the presence in several conjugants of 3, instead of the normal 2, gamete nuclei. The third gamete nucleus was located near the paroral cone but to one side of it while the other two were in the typical positions for male and female gamete nuclei. This observation suggests that occasionally two instead of one product of meiosis survive long enough so that they might take part in fertilization.

EXPERIMENTAL

Haploid × diploid crosses

The combined results of the crosses of 19b and 3b with diploid CD are shown in table 1. Two main facts can be seen by superficial examination. There are a large number of clones with poor growth, and there are a number of clones which appear to have obtained one of the markers from the haploid but not the other. This is the result expected if the single chromosome set in the haploid is segregated more or less at random to the meiotic products with the result that marker genes are lost and there is extensive aneuploidy in the hybrids.

However, this conclusion cannot be accepted without further consideration of the validity of phenotype determinations. For clones with good growth, the data in table 2

TABLE 1

Results of crosses between cl/cl d^{60}/d^{60} *diploids and* cl^{+} d^{90} *haploids*

Line	Clones from:				Clonal growth characteristics‡				
	Diploid conjugant		Haploid conjugant		Number of pairs of clones				
	Phenotype†		Phenotype						
	Crystals	Serotype	Crystals	Serotype	G/G	G/P	P/G	P/P	Total
1	+	60 and 90	+	60 and 90	0	0	0	7	7
2	+	60 and 90	−	60 and 90	0	0	0	2	2
3	+	60	+	60 and 90	0	0	0	4	4
4	+	60	+	60	0	0	0	3	3
5	−	60 and 90	+	60 and 90	0	1	0	4	5
6	−	60 and 90	−	60 and 90	0	0	0	2	2
7	+	60	+	90	0	0	0	1	1
8	−	60 and 90	+	90	0	0	0	1	1
9	+	60 and 90	?	?	0	0	0	1	1
10	−	60 and 90	?	?	0	0	0	6	6
11	+	60	+ or ?	?	0	1	0	1	2
12	+	?	+	60 and 90	0	0	0	7	7
13	+	?	+	90	0	0	0	2	2
14	+	?	+ or ?	?	0	1	0	18	19
15	−	60	+	60 and 90	2	8	0	1	11
16	−	60	+	60	0	4	0	0	4
17	−	60	−	60 and 90	0	1	0	0	1
18	−	60	−	60	1	2	0	1	4
19	−	60	+	90	2	7	3	0	12
20	−	60	+, −, or ?	?	0	28	0	7	35
21	− or ?	?	+	60 and 90	0	0	2	5	7
22	?	?	−	60 and 90	0	0	0	1	1
23	?	?	−	60	0	0	1	0	1
24	?	?	+	90	0	0	1	2	3
25	− or ?	?	+, −, or ?	?	0	0	0	32	32
Total									173

† +, crystals present; −, crystals absent. 60 means reacted with anti-60 serum; 90, reacted with anti-90 serum; 60 and 90, reacted with both sera. ? means that the phenotype could not be ascertained either because the clone died before 4 days or, rarely, because of an inconclusive observation.

‡ G means that the clone attained maximum population in 4 days; P, that it did not. The symbol to the left applies to the clone from the diploid conjugant; the symbol to the right, to the clone from the haploid conjugant.

show that the phenotype for crystals and serotype is an accurate indicator of the genotype. The table gives the results of a cross between stock CD and a diploid heterozygous for *cl* and *d*. The four main phenotypic classes occur in frequencies which do not differ significantly from the expected equal numbers, and the two members of the pair are alike except for the one aberrant pair. The regularity of the results demonstrates that the markers function satisfactorily in clones with good

TABLE 2

Cross of CD (cl/cl d^{60}/d^{60}) *with a heterozygous* ($cl^+/cl\ d^{60}/d^{90}$) *diploid*

Phenotype of clones from:*				No. of pairs
CD conjugant		Heterozygous conjugant		
Crystals	Serotype	Crystals	Serotype	
+	60 and 90	+	60 and 90	49
+	60	+	60	43
−	60 and 90	−	60 and 90	53
−	60	−	60	34
−	60	+	60	1
Total				180

* Symbols are the same as in table 1. All clones grew well (G) except for the +60 clone of heterozygous cytoplasmic origin on the last line.

TABLE 3

Presence of the marker characters from the haploid conjugant in clones with marker characters from both parents (data from table 1)

Crystals*	Serotype*	Number of clones from	
		Diploid conjugant	Haploid conjugant
+	60 and 90	10	41
+	60	10	7
−	60 and 90	14	6

* Symbols as in table 1.

growth. However, the fact that the markers are satisfactory for clones with good growth does not demonstrate that they will be equally satisfactory for clones with poor growth. Evidence on this point is given in tables 3 and 4.

Table 3 summarizes the data from the clones in table 1 which were successfully scored for both crystals and serotype and seemed to possess markers from both parents. The *gain* of crystals and of the reaction with anti-90 serum by clones of CD cytoplasmic origin occurs in the frequency expected if there were random segregation of the two markers during meiosis in the haploid. Clones with both characters, with crystals only, and with the 90 specificity only occur in equal frequency. It would be expected that there would be about the same number of clones with neither marker, and in lines 15 through 20 of table 1 there are 12 clones with poor growth which have neither. The clones with good growth in these lines represent a separate problem to be considered presently. The close fit to expectation makes it probable that gain of these characteristics is complete or nearly so even in clones with quite poor growth.

A further check on this conclusion is provided by the data in table 4. The table shows the phenotypes of clones of CD cytoplasmic origin from a cross of a haploid derived from CD with a diploid of stock 90, the reverse of the crosses in tables 1 and 3. Of the 29 poorly growing clones which showed at least one marker from 90, only 4 failed to show the other. Three other clones showed neither marker but these may

TABLE 4

The phenotypes of clones of haploid cytoplasmic origin from a cross of cl d^{60} *haploids with* cl^+/cl^+ d^{90}/d^{90} *diploids*

Phenotype*			Number of Clones
Crystals	Serotype	Growth	
+	60 and 90	P	21
+	60	P	2
+	90	P	4
−	60 and 90	P	2
−	60	P	3
+	60 and 90	G	2
+	90	G	1
Dead within 4 days or incompletely classified			55
Total			90

* Symbols are the same as in table 1.

have obtained no nucleus at all from 90. In this cross, any clone which obtained a nucleus from 90 should show both markers unless phenotypic lag prevented it. Thus it can be calculated that between 84 and 93 percent of the clones show the phenotype corresponding to their genotype in respect to the markers gained from the 90 parent.

The situation is much less favorable for detecting loss of the markers. The second column in table 3 shows that most of the clones from the haploid parent have the phenotype determined by both cl^+ and d^{90} even though it would be expected that half would lose one or the other. Similar conclusions can be drawn for d^{60} from table 4. On the assumption of random loss of markers from about half the clones, it can be calculated from tables 3 and 4 that loss of markers will be detected in only about a quarter of the cases in which it occurs. This conclusion is based on the assumption that marker genes are equally likely to be missing from the male and female gamete nuclei of the haploid. Other explanations of the data cannot be completely excluded. For example, the extra gamete nuclei, which were observed cytologically, may, by multiple fusion, make loss of markers less likely in the female contribution to the synkaryon. However, the phenotypic lag explanation seems quite probable and will be accepted in the rest of the discussion.

One other possible difficulty with the markers can be eliminated. A considerable number of poorly growing clones have been obtained both by autogamy in haploids of CD origin and by autogamy in irradiated clones of CD. In no case have these poor clones developed crystals. Thus the conditions causing poor growth are not sufficient to cause crystals to be formed in animals which lacked them before fertilization. The cl^+ allele must be introduced.

With this information on the markers, it is possible to draw conclusions on certain aspects of nuclear behavior from the data in table 1. The clones of diploid cytoplasmic origin in lines 1 through 14 of the table are all of the phenotype expected of hybrids

between the two parents. The lag phenomenon makes it impossible to establish beyond doubt that the clones received a nucleus from the diploid parent, although this seems quite probable. However, it is established that they obtained a male gamete nucleus from the haploid and that this nucleus frequently lacked one or the other of the marker genes. In most cases, the clones grew poorly as would be expected if they were extensively aneuploid; but in three cases there was good growth, at least in the first 4 days. Two of these latter clones lacked a marker gene and so were probably aneuploid for at least one chromosome. Random segregation of the approximately 40 pairs (DIPPEL 1955) of chromosomes would not be expected to give rise this frequently to clones with only one chromosome missing. Non-random segregation, selection of more complete meiotic products, resistance to the deleterious effects of aneuploidy, the presence of inert chromosomes or probably some combination of these is required. All that can be said at this time is that the chromosomes bearing *cl* and *d* appear to segregate at random and nuclei lacking them do not suffer a marked selective disadvantage compared to nuclei containing them but lacking other chromosomes. Thus the information on the male gamete nucleus from the haploid is in general agreement with a more or less random distribution of the single chromosome set to the meiotic products, but certain departures from this simple situation probably occur.

The G clones in lines 15 through 20 of the table show that the haploid often fails to contribute any detectable nucleus to the diploid mate. These clones grew well and, in addition to lacking crystals and reacting only with anti-60 serum, were dumpy and mating type I as would be expected if they had obtained nuclei only from CD. If it is assumed that all clones of diploid origin which grew poorly received a nucleus from the haploid, then the clones which did not receive such a nucleus make up about ⅓ of all pairs, It can be seen from table 1 that many of these pairs were true conjugants since the d^{60} allele was contributed to the haploid mate. Thus there is good evidence that failure of the male gamete nucleus of the haploid is a specific aberration of the conjugation process. However some pairs, especially those in line 19, may have been cytogamous or non-conjugants.

The table also shows that the diploid contributed a male gamete nucleus to the haploid in a large fraction of the cases in which sufficient evidence is available. A reaction with anti-60 serum was found in 59 of the 78 clones for which the serotype was determined. Actually the 6 G clones of haploid origin in lines 19 and 24 were probably macronuclear regenerates or non-conjugants and so should not be included in the critical cases. Moreover on the basis of the data for d^{90} in table 4, about 10 percent of the clones which obtained d^{60} should fail to show it because of lag. When these corrections are made, it can be calculated that in about 90% of all pairs in which a test was possible, the diploid contributed a male gamete nucleus to the haploid.

It can also be shown that the female gamete nucleus from the haploid member functions much more frequently than the male. This cannot be demonstrated directly by the marker genes because phenotypic lag introduces too much uncertainty. However, most of the clones which were of haploid cytoplasmic origin, grew poorly, and reacted with anti-60 serum were almost surely hybrids. The reaction with

TABLE 5

Comparison of the results of conjugation between diploids and diploids, haploids, or haploid-diploids with and without about 5 kr of X-rays

Classification of pair for growth and crystals*	Number of pairs as percentage of total number						
	CD × 90 diploid × diploid		CD × 3b or 19b diploid × haploid		CD × 20b diploid × haploid-diploid		
	0 kr	5 kr‡	0 kr	5 kr‡	0 kr	5 kr‡	E†
G+/G+	93.7	85.9	0.0	0.0	90.4	27.1	28.6
G−/any	2.2	9.6	31.8	28.4	6.2	32.9	22.2
Other	4.1	4.4	68.2	71.6	3.4	40.0	49.2
Total number of pairs	270	270	173	88	177	85	

* (Symbols as in table 1). The clone from the CD (diploid) member is shown to the right; the clone from other member, to the left.

† Distribution expected if one third of the pairs had the distribution of the diploid × 5 kr diploid group and two thirds had the distribution of the diploid × 5 kr haploid group.

‡ In each case the CD member was not irradiated and the other member was irradiated at least one day prior to conjugation.

anti-60 serum shows that they obtained a gamete nucleus from the diploid and if this were the only source of their nuclei they should have been simple haploids which normally grow well. The poor growth is evidence for aneuploidy resulting from a nuclear contribution from the haploid parent.

Haploid-diploid × diploid crosses

The mixed haploid-diploid clone 20b was crossed with CD, and the results are tabulated in table 5. Crosses between two diploids and between diploids and haploids are shown for comparison. The cross with unirradiated 20b gave the same results as the cross between two diploids. However, the cytological observation that one diploid and two haploid nuclei were present is confirmed by the cross of irradiated 20b with unirradiated CD. The results are very nearly those expected if one third of the pairs were like those from the cross of the unirradiated with the irradiated diploid and two thirds like those from the cross of the unirradiated diploid with the irradiated haploid. Thus the diploid nucleus of unirradiated 20b functions almost to the exclusion of the haploid nuclei, but this selective advantage is essentially eliminated by 5 kr of X-rays. This finding will be discussed further in the second paper of this series and is introduced here only to demonstrate that the nuclear constitution of 20b is the one assigned it by microscopic examination. This is also supported by the observation that about half of the poorly growing clones from the CD member of the cross with irradiated 20b had crystals, and half did not as would be expected if they were aneuploids derived from meiotic products of the haploid nuclei.

DISCUSSION

There are at least the following steps between meiosis and synkaryon formation: (1) entrance of a meiotic product into the paroral cone, (2) disintegration of the

meiotic products outside the cone, (3) division of the cone nucleus to form the gamete nuclei, (4) exchange of male gamete nuclei and (5) fusion of male and female gamete nuclei. SONNEBORN (1955) has shown that one factor in the first step is the expansion of the macronucleus as it breaks up into a skein. A clone (d59) in which this breakdown occurred less rapidly than usual failed to get any meiotic products into the cone. This evidence would be consistent with the view that a nucleus was carried more or less passively into the cone. However, the very fact that conjugation proceeds normally in the overwhelming majority of pairs between normal clones suggests a more active attraction into the paroral cone; otherwise nuclei should frequently fail to enter. This is probably implied by SONNEBORN's use (1955) of the phrase "sphere of influence" of the paroral cone.

The crosses with 20b show that the nature of the individual micronuclei or their products is also of importance. The cytological observations show no clear difference between the haploid and diploid nuclei in the rate at which they pass through the earlier stages of meiosis. The observations of normal diploids show that there is sufficient variation in position and timing so that differences which were not readily detected at these stages would be expected to be without any appreciable effect. They would be lost in the general random variations of the process. Thus the difference in behavior probably occurs during the very last stages of meiosis or in the postmeiotic period.

One explanation which, at first thought, is appealing is that chromosome bridges formed by non-homologous crossing over in the haploid are involved. However, this seems most improbable. If such bridges were able to nearly exclude the haploid nuclei from participation in fertilization, they should appreciably delay both meiotic divisions in almost all haploids. No evidence for this was found. Moreover, cytological observations of normal diploids suggests that there is an interphase following the second meiotic division during which considerable movement of the meiotic products occurs. Thus even if bridges slightly delayed this division, the delay would probably have little effect on the position of the nuclei at the time of entrance into the cone. In fact, if anything, it should bring the delayed nuclei nearer to the cone not further away since products of the first meiosis often start their second division in the general vicinity of the cone. Finally, it seems unlikely that chromosome bridges would have any appreciable effect on the separation of daughter nuclei because the spindles are very large compared to the chromosomes and most of the separation of daughter nuclei appears to involve an extensive elongation and attenuation of the central part of the spindle.

Thus it seems very probable that the failure of the haploid nuclei to contribute to fertilization in the cross with unirradiated 20b is to be attributed to the genetic deficiency of their meiotic products and not to the original haploidy or to mechanical difficulties imposed by chromosome bridges.

The second step, death of nuclei outside the cone, has been attributed by SONNEBORN (1955), on the basis of the behavior of d59, to a lethal effect of the cytoplasm on nuclei lying outside the cone. The cytological evidence from the present work for survival of meiotic products outside the cone suggests that the region in which nuclei can be located and survive is probably somewhat larger than the cone itself.

In normal conjugation, only one nucleus remains near the cone; but in haploid conjugation, more than one may stay near it, presumably because of defective nuclear behavior. This hypothesis agrees with the cytological observation that extra nuclei, when found, are near the cone, and it does not require the improbable assumption that the genetically deficient nuclei are less likely to be killed than normal ones.

It has been shown that the haploid frequently fails to contribute a male gamete nucleus to its diploid mate, although it may contribute a functional female nucleus to itself. A number of explanations of this observation can be suggested, and several examples will be given here: (1) no nucleus reaches the cone, but at least one outside the cone survives and functions as a female nucleus; (2) a deficient cone nucleus fails to divide and remains behind as a female nucleus; (3) the cone nucleus fails to form a functional gamete nucleus of either kind, and the female nucleus comes from a nucleus surviving outside the cone; and, (4) the cone nucleus forms both male and female nuclei, but the former fails to reach the female nucleus of the normal mate. The evidence for competition for entrance into the cone provided by the cross with 20b makes (1) the least probable since, with many nuclei available, it is quite unlikely that all could fail to reach the cone. The other explanations do not involve this difficulty, and attribute the failure of the male gamete nucleus to defective behavior of the cone nucleus itself. The differential failure of the male gamete nucleus in these various schemes is attributed either to a greater complexity of the activity of the male nucleus making it more likely to fail than the female, or to a greater number of nuclei which can serve as a source of the female nucleus so that when one source, the nucleus in the cone, fails other sources are often available.

Whatever the precise nature of the failure, the same arguments which were used in connection with entrance of a meiotic product into the cone can be used to support the view that it is the genetic constitution of the meiotic products themselves which interferes with the formation of a functional male nucleus. However, it is necessary first to exclude the possibility that there is some effect of haploidy on the whole animal which then interferes with the formation of the gamete nucleus. In the first place, cytological evidence suggests that the macronucleus is the same size in haploids and diploids. In other words, the degree of polyploidy reached is not dependent on the number of chromosomes in the nucleus which gives rise to the macronucleus. The major characteristics of the whole animal are determined by the macronucleus and so it is unlikely that haploidy of only the micronuclei would have any general effect. In the second place, the results of irradiation of 20b are very difficult to understand on this basis. The fraction of pairs in which the male gamete nucleus from irradiated 20b failed to function is not significantly different from that expected if the haploid nucleus contributed gamete nuclei $\frac{2}{3}$ of the time. If a systemic effect of the radiation were involved, it would have to be assumed that somehow it duplicated rather precisely the results expected if only the nuclear constitution of the meiotic products was important. This possibility seems very remote, especially since the same dose of radiation had practically no effect on the formation of male nuclei by ordinary diploids and haploids (see table 5).

On the basis of the preceding argument, it can be concluded that the behavior

of nuclei involves localized interactions of the nucleus' own genome with the immediately surrounding cytoplasmic environment. The latter may, of course, be under the control of other nuclei within the common cytoplasmic mass. The deficient nature of the meiotic products of haploid micronuclei cannot be adequately supplemented by products diffusing to them from other nuclei in the same cytoplasm and so their behavior is, in this respect, autonomous. This situation contrasts with the more usual finding with heterokaryons that genetically diverse nuclei in a common cytoplasm complement each other and act as though the diverse genomes were contained in a common nucleus. However, there is some evidence in heterokaryons of fungi that the distribution of the parts of the total genome to the various nuclei may be of importance though the cases are not entirely similar. PONTECORVO (1952) discusses this and other problems concerning the localization of nuclear processes within the cell.

SUMMARY

1. Crosses were made between a diploid marker stock of *Paramecium aurelia* and clones having haploid or both haploid and diploid micronuclei and conclusions were drawn from the genetic and some cytological data about the behavior of the meiotic products of the haploid.

2. In conjugation between haploids and diploids, the diploid usually contributes a male gamete nucleus to the haploid, but the haploid frequently fails to contribute a male nucleus to the diploid. However, even in this latter case, the haploid generally contributes a female gamete nucleus to itself.

3. The gamete nuclei of the haploid are usually grossly deficient as a result of more or less random segregation of the single chromosome set at meiosis.

4. When both haploid and diploid micronuclei are present in a common cytoplasm, the gamete nuclei almost always come from the diploid. This selective advantage can be almost completely eliminated by 5 kr of X-rays.

5. The two major abnormalities in the nuclear behavior of the meiotic products of haploid micronuclei are, therefore, (1) a frequent failure to form a functional male gamete nucleus and (2) an inability to compete successfully with products of diploid micronuclei for the formation of gamete nuclei.

6. Reasons are given for believing that these abnormalities are the result of the deficient genome of the meiotic products and that nuclear behavior is controlled by interactions between the nucleus' own genome and the surrounding cytoplasm.

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COMPARATIVE STUDIES OF MONOEPOXIDES AS INDUCERS OF REVERSE MUTATIONS IN NEUROSPORA[1]

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IN two previous papers (KØLMARK and WESTERGAARD 1953; KØLMARK 1953) experiments have been reported dealing with studies of epoxides as mutagens. In these experiments the induction of reverse mutations, principally in a purple adenineless mutant of *Neurospora crassa*, served as the criterion of mutagenic activity for the various compounds tested. Certain of these compounds proved to be very potent mutagens. The general results suggested that additional comparative studies of related substances should prove fruitful in elucidating certain aspects of the mechanism of action of these chemical mutagens. In the present paper the results of comparative studies of six different monoepoxides as inducers of reverse mutation in a purple adenineless mutant will be presented.

MATERIALS AND METHODS

The monoepoxides used in these experiments were obtained from the following commercial sources: from Eastman Kodak Chemical Corporation—glycidol, propylenoxide, 1,2-monoepoxybutane, 2,3-monoepoxybutane; from Farchan Research Laboratories—epichlorohydrin and epibromohydrin. All these compounds are liquids at room temperature.

The general method employed in testing various chemicals for mutagenic activity by the back-mutation technique has been described previously (KØLMARK and WESTERGAARD 1949; JENSEN, *et al.* 1951). In the present experiments the test culture, W. 40 "distinctus" A (an extreme colonial strain of purple adenineless mutant 38701), and the methods of chemical treatment and reversion detection have been the same as those utilized in the initial experiments with epoxides (KØLMARK and WESTERGAARD 1953). The various chemicals are added directly to suspensions of macroconidia in sterile distilled water, utilizing a micropipette attached to a screw syringe. The conidial suspensions are kept at 25°C and gently agitated during the period of treatment. The treatment is stopped by centrifuging the conidia and washing several times with sterile water. The conidia are then plated at appropriate concentrations into a minimal agar medium. The numbers of viable and of surviving conidia in the control series and in the treated series respectively are determined by plating diluted samples on minimal medium supplemented with adenine.

When starting to test a chemical whose toxicity for Neurospora conidia is unknown, the usual initial procedure is to perform an experiment in which the concentration of the chemical is varied over a wide range, the time of treatment being kept constant.

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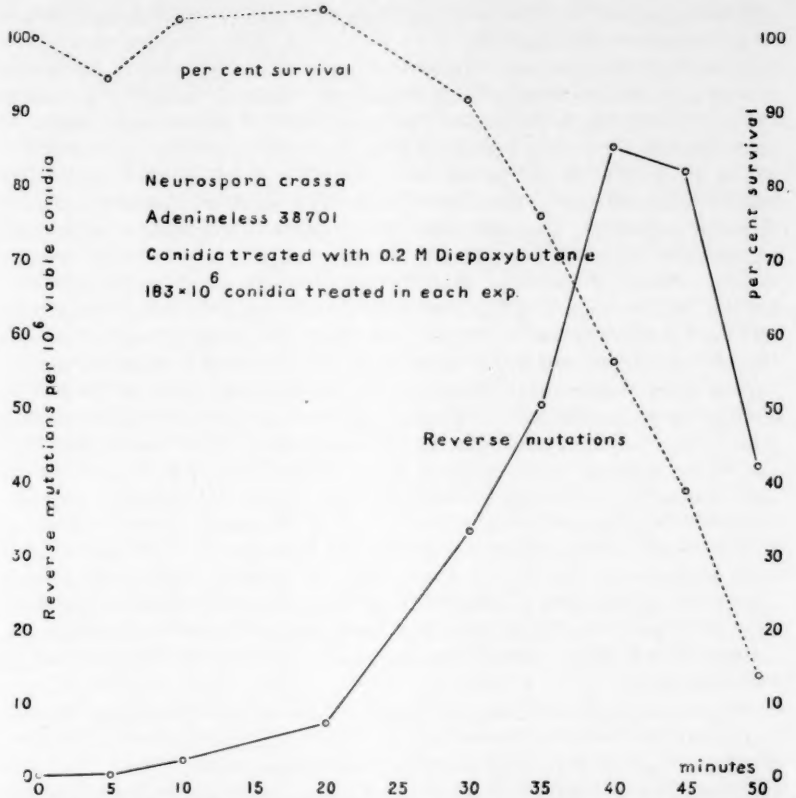


FIGURE 1.—The effect of exposures of macroconidia of purple adenineless mutant 38701 (strain W 40 "distinctus" A) to 0.2 M diepoxybutane for varying lengths of time. The lower curve gives the frequency of reverse mutations, the upper, percent conidial survival, based on the value of 100 percent in the control.

From such an experiment a concentration will usually be found which produces a considerable conidial killing within a time convenient for the experiment, for example, 50 percent killing in 45 minutes. This concentration can then be used in a second experiment in which the time of exposure is varied. Figure 1 illustrates the type of curve which is obtained in an experiment in which a constant concentration of a chemical has been utilized and the time of treatment varied. The data on which this figure is based are those obtained by KØLMARK and WESTERGAARD (1953) with diepoxybutane, the chemical mutagen having the greatest activity of any so far tested in producing reversions in purple adenineless mutant 38701. This curve may be used in comparing the activity of this diepoxide with the various monoepoxides tested in the present experiments. It will also prove useful in discussing the concept of

"optimal conditions" which is used in making comparisons of the mutagenic activities of the various compounds tested.

In the experiment plotted in figure 1, diepoxybutane was added to the conidia at time zero. Aliquots were removed at intervals, the reaction stopped by washing the conidia with distilled water, and the conidia plated to determine the number of reversions and the percent conidial survival. Each point represents a treatment of 183×10^6 conidia. In the control there were a few spontaneous reversions (the number was 5). With increasing time of exposure the number of induced reversions increased as shown in the lower curve until it reached a maximum at 40 minutes treatment, at which point more than 15,000 reversions were scored. In the curve the derived numbers of mutations per 10^6 treated initially viable spores have been plotted. The slow rise in the number of reversions during the first 20 minutes of treatment is probably due to the time required for the chemical to penetrate into the cell and into the nucleus. The upper curve gives the percent conidial survival at various times. A pronounced mutagenic effect is observed before the toxic effect becomes significant. However, this time difference in toxic and mutagenic activity is presumably only an apparent one. Since macroconidia have been treated, more than one nucleus is present in the majority of the conidia. Hence it is expected that in most instances each conidium containing a nucleus which has undergone reversion to the non-requiring state will give rise to a colony on minimal medium, whereas in most instances the occurrence of a single lethal mutation in a conidium will not result in the death of the conidium. For example, if a conidium contains three nuclei, a reversion in only one nucleus is required to give rise to a colony on minimal medium, whereas at least three lethal mutations are presumably needed to prevent this conidium from forming a colony on minimal plus adenine medium where the viability count is made.

The maximal yield of mutations is obtained after an exposure which gives approximately 56 percent survival. Beyond this point, the total yield of reversions begins to decrease because the toxic effect increases so rapidly that conidial killing occurs at a faster rate than reversion production. When a particular mutant, such as this purple adenineless is treated with a chemical, such as diepoxybutane, the experimental conditions giving the highest yield of mutations may be designated the "optimal conditions." The relative mutagenic activities of the various monoepoxides used in the present experiments have been compared on the basis of their effectiveness under "optimal conditions" for each mutagen. It is realized that this basis of comparison is not a perfect one. In some respects it would appear desirable to compare the various chemicals on an equimolar basis. Unfortunately, since toxic effects vary markedly from one chemical to another, such comparisons are often difficult if not impossible. It appears, however, that even if the molar concentrations are rather different, the percent survival under "optimal conditions" is usually rather similar. Hence, to a considerable degree the present comparisons are being made at equal or similar survival levels.

Clearly, there are many variables involved in determining how effective a particular chemical will be as a mutagen. Some of these variables have been discussed by KØLMARK and WESTERGAARD (1953) and it is apparent that numerous factors other

than relative chemical affinity for a particular gene may influence the mutagenic effectiveness of structurally similar compounds. Although we are aware of these difficulties, it has seemed desirable in these initial comparative experiments to make a first attempt at establishing the order of mutagenic activity of the monoepoxides being studied. Because of the great differences in activity found even between closely similar compounds, it appears likely that these indicate basic differences in the mutagenic effectiveness of these chemicals with respect to this adenineless mutant. The relative effectiveness of these compounds as determined by the experimental procedures outlined will be used as the basis for a discussion later in this paper of a possible mechanism of mutation induction by monoepoxides.

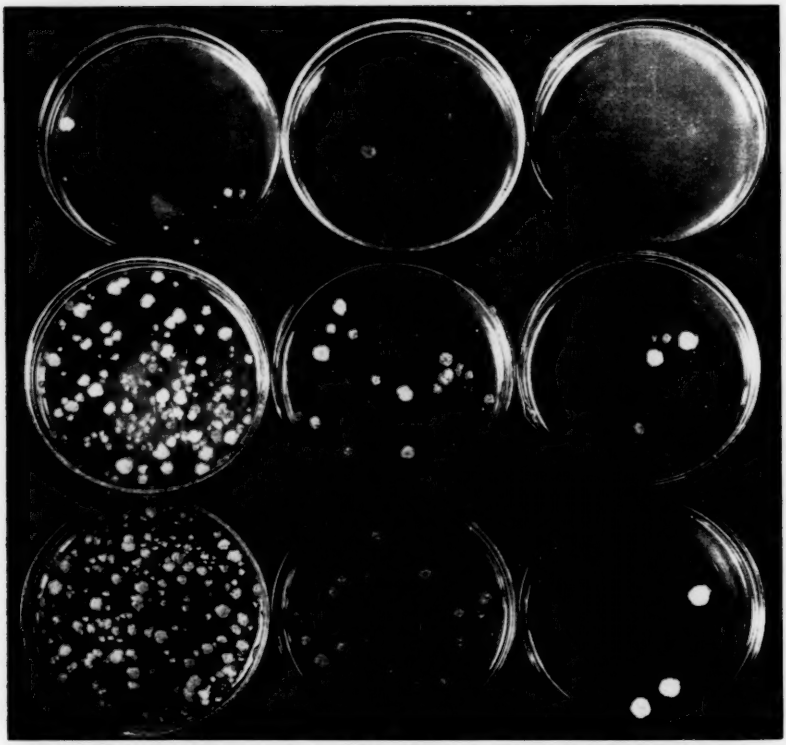
In connection with the experiments performed to determine the mutagenic activity of the various compounds, control platings were carried out to ascertain that no competition effects were present resulting in the selective suppression of reversions under particular conditions (cf. GRIGG 1952; KØLMARK and WESTERGAARD 1952). Representative results of one such series of platings are given in table 1 and illustrated in figure 2. It is clear that there is no disproportionate increase in reversion frequencies with either a 10- or 100-fold conidial dilution.

EXPERIMENTAL RESULTS

The quantitative data obtained in experiments with the various monoepoxides are given in tables 2–5. A detailed consideration of these results will be deferred until the discussion.

Because of the marked differences in the mutagenic effectiveness of the various compounds, it seemed especially desirable to make genetic tests of a number of reversions to determine whether suppressor rather than reverse mutations might have occurred. In most instances, reversions appearing on minimal are not only adenine-independent, but no longer form purple pigment. In some experiments with monoepoxides, however, a number of colonies appeared on minimal which still produced some purple pigment. It seemed possible that these might be the result of suppressor mutation. Tests of a number of these purple revertants revealed, however, that they were heterocaryons. In the majority of cases both revertant (adenine-independent, non-purple) and non-revertant (adenineless, purple) components could be separated by conidial platings. In a few cases no non-requiring colonies were obtained by diluted conidial platings on minimal medium. Only the very slight type of growth—called "shadows"—was observed. This type of growth is typical for this adenineless strain when only a few spores germinate on a plate with minimal medium. These latter cases apparently are due to heterocaryons with a great preponderance of requiring nuclei, so that occasionally no non-requiring nuclei have been transferred by the isolation. Since almost all reversions induced in macroconidia are heterocaryotic, pigment expression is not always evident and apparently occurs when the nuclear ratio favors adenineless nuclei.

Crossing analyses were carried out with a total of 38 independent reversions arising spontaneously or induced by four of the monoepoxides tested. The results of these genetic tests are summarized in table 6. Most of the reversions were induced in the macroconidiating strain W. 40 "distinctus" A. In all such instances, the revertants



Spores undiluted diluted 10× diluted 100×

Reverse mutations in *Neurospora crassa*, purple adenineless, 38701, macroconidiating, "distinctus"
Upper row: control, untreated.
Middle row: 0.5 M. propylenoxide, 15 min.
Lower row: 0.5 M. glycidol, 15 min.

FIGURE 2.—Photographs of representative plates from a dilution series containing reverse mutations induced in purple adenineless mutant 38701 by two epoxides. See table 1 and text for further discussion.

were first outcrossed to a strain of purple adenineless 38701a and homocaryotic isolates obtained for crossing with wild type (strains 74A or 73a). In some instances reversions induced in a microconidiating strain (8743-CL20-12.1a) were tested and these were crossed directly to wild type. In all cases the progeny from crosses of homocaryotic reversions with wild type were scored for the presence of typical purple pigment. The number of such isolates tested ranged between 24 and 196 for 38 crosses, the average number being 90. In no instance was a culture with typical purple pigment found. In addition, in a number of crosses, all the progeny were also tested on minimal medium, and no instances of adenineless segregants were obtained.

TABLE 1

Evidence for the absence of a suppression effect of high conidial concentrations on the recovery of reversions as demonstrated by the effect of conidial dilution on the frequencies of reversions after treatment with two epoxides. 132 × 10^6 viable macroconidia treated in each experiment. See text for further discussion

Treatment	No. of colonies counted on five plates each at the conidial dilutions indicated		
	Undiluted	10^{-1}	10^{-2}
Control	8	3	1
0.5 M Propylenoxide			
15 min.	780	77	8
30 "	1647	156	17
45 "	ca. 2500	271	—
60 "	ca. 2750	282	—
0.5 M Glycidol			
15 min.	1025	101	9
30 "	ca. 2000	195	17
45 "	ca. 3500	349	—
60 "	ca. 4250	445	—

TABLE 2

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments with 0.5 M propylenoxide or 0.5 M glycidol for varying lengths of time. 132 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	8	0.0	0.0
	0.5 M Propylenoxide				
II	15 min.	94.0	780	5.9	6.2
III	30 "	83.5	1647	12.4	14.9
IV	45 "	63.1	2710	20.5	32.5
V	60 "	26.7	2820	21.4	80.0
	0.5 M Glycidol				
VI	15 min.	92.0	1025	7.7	8.4
VII	30 "	85.7	1950	14.7	17.4
VIII	45 "	62.3	3490	26.4	42.5
IX	60 "	26.3	4450	33.8	128.5

* The frequencies have been corrected for the spontaneous background.

These results indicate that the majority of reversions induced by these chemicals do not involve suppressor mutations, unless the latter are quite closely linked with the purple adenineless locus. Hence it appears reasonable to compare the various epoxides on the basis of their relative effectiveness as inducers of reverse mutation in the purple adenineless mutant.

TABLE 3

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments for 30 minutes with varying concentrations of either 1,2-monoepoxybutane or 2,3-monoepoxybutane. 234 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	29	0.0	0.0
	1,2-Monoepoxybutane, 30 min.				
II	0.005 M	100	32	0.013	0.013
III	0.025 M	56.9	36	0.09	0.16
IV	0.050 M	57.3	91	0.32	0.55
V	0.10 M	52.2	239	0.96	1.8
VI	0.20 M	34.5	506	2.1	6.1
	2,3-Monoepoxybutane, 30 min.				
VII	0.05 M	67.5	25	0.021	0.032
VIII	0.10 M	ca. 50	32	0.07	ca. 0.15
IX	0.20 M	ca. 40	35	0.10	ca. 0.25
X	0.40 M	28.8	10	0.009	0.03

* The frequencies have been corrected for the spontaneous background.

TABLE 4

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments with 0.15 M epichlorohydrin for varying lengths of time. 73.6 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	39	0.0	0.0
	0.15 M Epichlorohydrin				
II	15 min.	94.7	626	8.0	8.5
III	30 "	87.8	2480	32.6	37.0
IV	45 "	41.5	4140	56.1	135.2
V	60 "	0.72	218	3.0	411.0

* The frequencies have been corrected for the spontaneous background.

TABLE 5

Reverse mutations induced in macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) by treatments for 30 minutes with varying concentrations of epibromohydrin. 71.3 × 10^6 conidia treated in each experiment

Expt.	Treatment	Percent survival	Reverse mutations		
			No. counted	No. induced*	
				Per 10^6 viable	Per 10^6 surviving
I	Control, untreated	100	10	0.0	0.0
	Epibromohydrin, 30 min.				
II	0.005 M	97.2	13	0.042	0.043
III	0.01 M	99.1	17	0.098	0.099
IV	0.02 M	85.5	30	0.29	0.34
V	0.04 M	77.5	65	0.80	1.0
VI	0.08 M	39.3	206	2.8	7.2
VII	0.16 M	0.0005	0	—	—

* The frequencies have been corrected for the spontaneous background.

DISCUSSION

On the basis of the data already presented, an attempt can be made to compare the relative mutagenic effectiveness of the six monoepoxides tested. To this end, figures on reverse mutation rates under "optimal conditions" for the various chemicals, derived from tables 2–5, have been collected in table 7, together with the structural formulae

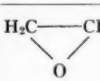
TABLE 6

Genetic analyses of reversions induced by various chemical mutagens in purple adenineless strain 38701. See text for further details

Mutagen	No. homocaryotic reversions tested	No. reversions giving typical purple segregants	No. reversions giving adenineless segregants
None (control)	7	0 in 7	0 in 3
Propylenoxide	12	0 in 12	0 in 6
Glycidol	4	0 in 4	0 in 4
1,2-Monoepoxybutane	4	0 in 4	0 in 3
Epichlorohydrin	11	0 in 11	no tests

TABLE 7

Relative mutagenic activity of six different monoepoxides. Comparisons have been made of reverse mutation rates under "optimal conditions" (see text). Figures obtained from the foregoing tables 2–5

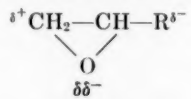
Chemical	Formula	Molar conc.	Percent survival	Reverse mutations induced*	
				Per 10^8 viable	Per 10^8 surviving
Epichlorohydrin		0.15	41.5	56.1	135.2
Epibromohydrin		0.08	39.3	2.8	7.2
Glycidol		0.5	26.3	33.8	128.5
Propylenoxide		0.5	26.7	21.4	80.0
1,2-Monoepoxybutane		0.2	34.5	2.1	6.1
2,3-Monoepoxybutane		0.2	ca. 40	0.10	0.25

* The frequencies have been corrected for the spontaneous background.

of the six monoepoxides. It is clear that there are marked differences in the comparative mutagenic effectiveness of the six compounds. Epichlorohydrin appears to be the most active mutagen, but both glycidol and propylenoxide are also quite active. Both the monoepoxybutanes are much less active, the 2,3 compound being especially ineffective.

As judged by the structural formulae included in the table these six compounds are all quite similar. Consequently, their marked differences in mutagenic effectiveness are of particular interest, since it seems possible that these differences may give some clue as the mechanism of action of these compounds in mutation production. Following a suggestion made by W. C. J. Ross of the Chester Beatty Institute, consideration of certain physico-chemical properties of monoepoxides has lead to the formulation of an hypothesis to explain the marked differences in mutagenic effectiveness already noted.

This hypothesis is based on the assumption that the reaction producing a mutation takes place between electrically charged entities. In the specific case as regards monoepoxides this reaction is assumed to occur between the positively charged terminal carbon atom of the epoxide ring and a negative center in the biological material, in this instance in the adenineless locus. Chemical evidence (Ross 1950) indicates that, although the epoxide ring is always polarized to some extent even when there are no substitutes, the presence of electron attracting (i.e., electronegative groups) in the side chain increases the positive charge on the terminal carbon atom and decreases the negative charge on the oxygen atom. Thus a monoepoxide molecule can be written as a dipole with a distribution of charges as follows:



Side chains which have stronger electronegative (electron attracting) properties will result in a relative increase in the positive charge on the terminal carbon atom of the epoxide ring. The side chains of the compounds used here are chemically rather inactive. The epoxyring, therefore, can be considered as the only active group in the molecule. Thus, if the mutation reaction involves a negative center in the genetic material, a monoepoxide with a more positively charged terminal carbon atom in the ring should be a more effective mutagen. The first two compounds tested with this idea in mind were propylenoxide and glycidol. The CH_2OH group in glycidol is more electronegative than the CH_3 group in propylenoxide. Thus, if the hypothesis outlined above is correct, glycidol should be the more effective mutagen. As indicated in table 7, glycidol does in fact produce about one and a half times as many mutations as propylenoxide. The results with the other monoepoxides generally support the hypothesis. In table 8 the groups in the side chains of the six monoepoxides have been arranged in order of decreasing electronegativity (reading from top of the table) and the mutagenic effectiveness of the compounds indicated.

Pairwise comparisons between the compounds most like each other give the following results: (1) The Cl-compound should be more effective than the Br-com-

TABLE 8

Six monoepoxides arranged in the order of decreasing electronegativity of their side chains (reading from top of table) and compared for mutagenic effectiveness

Monoepoxide	Side chain	Mutations per 10^6 viable conidia under "optimal conditions"
Epichlorohydrin	CH_2Cl	56.1
Epibromohydrin	CH_2Br	2.8
Glycidol	CH_2OH	33.8
Propylenoxide	CH_2H	21.4
1,2-Monoepoxybutane	CH_2CH_3	2.1
2,3-Monoepoxybutane	$2(CH_2H)$	0.1

pound, which accords with the results obtained; (2) The OH-compound should be more active than the H-compound, which is also the case; (3) A CH_3 side chain should be more active than a CH_2CH_3 side chain, which agrees with the results for propylenoxide vs 1,2-epoxybutane; (4) One CH_3 side chain should be more active than two CH_3 side chains, which also is the case in the comparison of propylenoxide and 2,3-monoepoxybutane.

If the order of the entire series is considered, the mutagenic activity of epibromohydrin is too low. On the basis of its position in the sequence it would be expected to yield more mutations than glycidol, which is contrary to the observations. This result, which is in disagreement with the working hypothesis, can perhaps be explained in part, if not entirely, by the greater toxicity of epibromohydrin, which makes difficult a direct comparison between it and the other substances. If a comparison is desired between two compounds with widely different toxicities, such as glycidol and epibromohydrin, used in equimolar concentrations, it is necessary to use a concentration of the more toxic substance which is low enough to be tolerated by the test organism. Consequently an attempt has been made to compare the activity of glycidol and epibromohydrin at lower equimolar concentrations, both compounds being used in 0.1 molar concentration. The results are shown in figure 3. Under these conditions the mutagenic and toxic effects of epibromohydrin occur very much faster than do those for glycidol.

On the basis of this experiment it would appear that epibromohydrin is the stronger mutagen of the two, which is in accord with our working hypothesis. However, it is also clear from this curve that the total number of mutations with epibromohydrin fails to reach a high value because of the toxic effect. Thus, the figure "mutations per 10^6 treated conidia" can never reach as high a value (33.8) as was found for glycidol when this compound was used at 0.5 molar concentration. Glycidol at 0.1 molar concentration acts very slowly as a mutagen and is far from its maximal effect after 120 minutes, at which time there is also little evidence of a toxic effect. If, in a comparison of this type, the same percent survival is desired, there will obviously have to be a very large time differential. If, by contrast, equal times of treatment are utilized, the percent survival will be very different for the two compounds. In view of

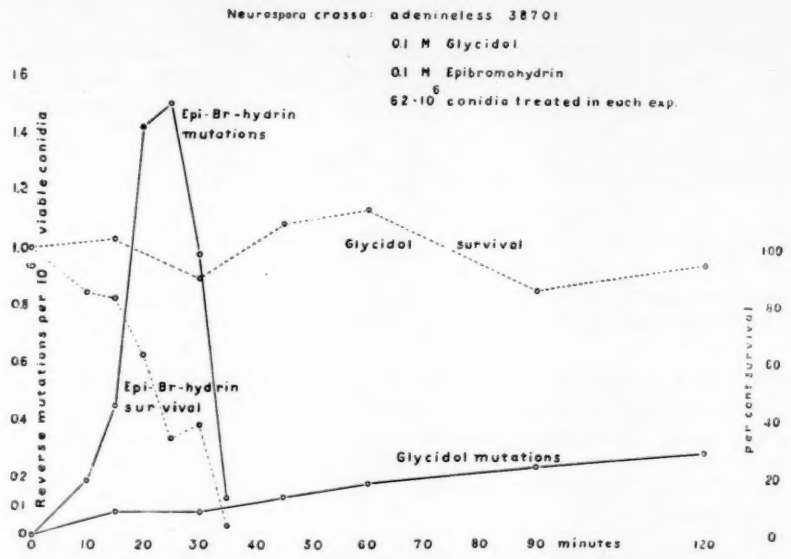
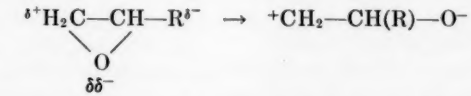


FIGURE 3.—The effect of exposures of macroconidia of purple adenineless mutant 38701 (strain W. 40 "distinctus" A) to 0.1 M glycidol and 0.1 M epibromohydrin for varying lengths of time. Mutation rates corrected for the spontaneous background. Percent conidial survival based on the value of 100 percent in the control.

these considerations it appears difficult to achieve a good basis for a comparison of the mutagenic activities of two compounds which differ considerably in toxicity. It is clear that comparisons between mutagenic activities based on the mutation rate under "optimal conditions" must be evaluated with caution if the compounds differ markedly in their toxicities. With these reservations in mind, it appears reasonable to conclude from these experiments that the possibility still remains open that epibromohydrin fits into the appropriate position in the sequence of activities.

The possibility has been mentioned by KØLMARK and WESTERGAARD (1953) that the mutagenic effect of epoxides might be due to a release or transfer of energy when the three-membered ring is opened. There is also the possibility that the product of a general chemical reaction gives rise to a mutation. Of course, it need not be that an initial chemical product between mutagen and gene be reduplicated in the induced mutant. Such a reaction might simply give rise to a steric disturbance in the normal gene during the first mitosis, the modified gene then being reduplicated without any alteration in its molecular size. The epoxides react with a variety of other groups to give alkylation compounds. Both these mechanisms still remain as possibilities if the epoxides are considered as dipoles—the electric charge might act merely to direct the mutagenic chemical to the location where a reaction will produce the particular mutation studied. Thus the actual mutagenic event might still be due to a chemical reaction or to a transfer of energy.

The different theories for biological reaction of radiomimetic agents have been reviewed recently by HOMER (1954). Our experimental results fall closely in line with one of the viewpoints cited in this paper which maintains that "all that is required for radiomimetic activity is that the drug shall give rise to a reactive positive ion, sometimes so unstable as to be formed only at the moment of reaction. . . ." Different types of radiomimetic compounds, including the epoxides, have the ability to form carbonium ions. The ionic form of an epoxide, created by opening the ring may be written:



which then can react further and, for example, form esters with carboxyl or phosphoryl groups. If such a preformed ion is involved in the mutagenic reaction, this will presumably be a chemical reaction. If, on the other hand, the ring is opened at the moment of mutation production, the mutation inducing mechanism might well be dependent on a simultaneous transfer of energy. It is not possible to decide which of these two possibilities is realized. The fact that a fractional positive charge is carried on the terminal carbon atom before the ring has been opened might favor the view that the compound is reacting directly from the ring structure at the critical time and place. Electrostatic forces would direct the positive terminal carbon atom to the place of the anion. This directing force would increase with the magnitude of the positive charge. Such a situation would lead to series of compounds with different activities as outlined in the working hypothesis and found in our experiments. We cannot provide any experimental evidence to identify the anion in the gene with which the epoxides presumably react, but it is tempting to think of phosphoryl groups in the backbone of DNA or carboxyl groups in nucleoproteins as the site of reaction.

The general conclusion of this investigation is that there exists a quite striking correlation between two properties of the epoxides used here: a high positive charge on the terminal carbon atom in the epoxyring and a high mutagenic activity. So far we know only that this correlation exists for reverse mutations in this particular adenineless strain (38701). We may in the future expect to find other relations with regard to other genes. Experiments with gene specificity (KØLMARK and WESTERGAARD 1953; KØLMARK 1953) already suggest that the correlations between mutagens and mutation rates will be different for different genes treated with the same mutagen.

For a given reverse mutation reaction, as in the work presented here, it appears that we can predict, by examining the formulae of the chemicals and taking into consideration their properties as dipoles, whether they will behave as strong or weak mutagens. On this basis it would appear desirable to study in future experiments epoxides having side chains with nitro (NO_2) and cyano (CN) groups. Such compounds would be expected to be more potent mutagens than any of the compounds yet tested. Also the sulphonic acid group (SO_3H) can be expected to increase mutagenic activity, but this effect will presumably be dependent on the pH of the solution as well (W. C. J. ROSS personal communication).

SUMMARY

1. Six different monoepoxides have been shown to induce reversions in the purple adenineless mutant (38701) of *Neurospora crassa*.

2. Genetic tests of reversions induced by four of these monoepoxides have been carried out. Only true reverse mutations and no suppressor mutations were found.

3. A comparison of the mutagenic effectiveness of the six epoxides has been made. This comparison is based on the relative number of reversions recovered per 10^6 viable treated spores when treatments have been performed under "optimal conditions." "Optimal conditions" are defined as the experimental conditions under which the highest yield of reversions is obtained.

4. The data indicate that in general epoxyrings carrying side chains with strong electronegative properties are stronger mutagens than compounds carrying weaker electronegative side chains.

5. On the basis of these experimental observations, a working hypothesis to explain the mutagenic effect of these compounds is developed and discussed. This hypothesis proposes that the mutation reaction involves negatively charged centers at the site of mutation which react relatively more readily with those epoxides carrying an increased positive charge on the terminal carbon atom of the epoxyring.

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LINKAGE IN UPLAND COTTON[1]

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ALTHOUGH the genetics of Gossypium has been studied intensively for more than thirty years, very few linkages have been reported. In the amphidiploid species to which the Upland (*hirsutum*) cottons of cultivation belong only four linkage groups have been identified. These are:

(1) Red (R_1)-cluster (cl_1) HARLAND (1939)
(2) Green Lint (Lg)-crinkle (cr) HARLAND (1939)
(3) Petal Spot (R_2)-short-branch (cl_2) SILOW (1946)
(4) Petal Spot (R_2)-yellow-green (yg_2) RHYNE (1955)

STEPHENS (1950) found a third locus, corky (ck) which was closely linked with crinkle, but its position relative to Green Lint was not determined. Two other possible linkages have been reported (HUTCHINSON 1946; KNIGHT 1952) but these have not been critically established. A possible linkage between lint color (Lc_2) and yg_2 was indicated by RHYNE (1955) but his data were incomplete.

The small number of linkages identified may be attributable partly to the fact that there are twenty-six pairs of chromosomes in the amphidiploid genome, and partly because no systematic search for linkage has been undertaken. Independent segregations, though often observed, have rarely been reported. Such reports as exist are subordinate to publications of work carried out for a different purpose, and as a result the existing information is widely scattered through the literature. At the present time there is considerable interest in the comparative cytogenetics of the amphidiploid cottons and their diploid relatives. These studies are at present limited by the small number of suitably marked stocks which are available as testers. Genes which are suitable as markers have been maintained in separate stocks; for systematic cytogenetic analysis it is desirable to have them available in relatively few, "multiple marker" stocks with linked genes incorporated in the most convenient (coupling or repulsion) phases for testing.

For several years the author has been engaged in synthesizing multiple marker stocks, and in the process, families have become available which are segregating simultaneously for a large number of marker genes. It has been possible to use this material in a systematic search for linkages. As a result, five new linkages have been discovered and a number of independent loci established. Both are important in providing a basis for future mapping of the amphidiploid genome.

MATERIALS AND METHODS

Most of the data to be reported were obtained from crosses involving marker genes already available in Upland stocks. A small number of segregations from inter-

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TABLE 1

List of loci tested for independence. The mutant form of the locus (i.e. as contrasted with the "normal" form found most commonly in cultivated Upland stocks) is listed in each case

Symbol‡	Name	Reference or description
cl_1	cluster	H & S†
cr	crinkle	H & S
*cn**	crenate	Sometimes called "round leaf". Very variable in expression, lobes of leaf being irregular, reduced or absent.
*cu**	cup leaf	LEWIS (unpub.). Phenotypically similar to Asiatic "curly" (H & S)
f_g*§	frego-bract	GREEN (in press).
H_2	Pilose	SIMPSON (1947); KNIGHT (1952).
L	Narrow Leaf	H & S
Lc_1	Brown Lint	H & S; RICHMOND (1943); BRAIN (1950). Includes "Nankeen", "Algerian" etc. allelic series (WARE unpublished).
Lc_2*	Brown Lint	Includes at least two distinct brown alleles, "Higginbotham" and "Brymer" as described by WARE (1932) and BRAIN (1950).
Lg	Green Lint	H & S
N	Naked Seed	The symbol, *N*, is in common usage and is therefore preferred to *Fn* in H & S nomenclature.
P	Yellow Pollen	H & S
R_1	Red Plant	H & S
R_2	Petal Spot	H & S
Rd	Dwarf Red	JOHNSON (1949)
v	virescent yellow	H & S
Y_1	Yellow Petal	H & S
yg_2	yellow-green	RHYNE (1955)

* Proposed new symbol.

† H & S refers to HUTCHINSON and SILOW (1939).

‡ Superscripts omitted from symbols.

§ The symbol *Fg* was proposed by H & S to indicate Green Fuzz. Since Green Fuzz appears to be an allele of Green Lint, *Lg*, (STEPHENS unpublished), the symbol *Fg* is no longer applicable to this character.

specific hybrids was also studied. The marker loci tested are listed in table 1. Genes previously reported in the literature are given the symbols assigned to them by HUTCHINSON and SILOW (1939) except that superscripts have been omitted. Their system of nomenclature is rather impractical but no suitable revision has yet been attempted.

In the primary screening for linkage groups, backcrosses were always employed, since these provide more reliable information per unit of family size. For economy of space the complete four class segregation for each recombination studied is not presented. By listing only the percentage recombination and family size, it is possible to summarize the results in a tabular form (fig. 1). For convenience in evaluating the results the maximum crossover values which are distinguished from independence in a given family size at the 5% and 1% levels of significance are listed in table 2. These are calculated as follows:

	cr	cn	cu	fg	H_2	L	Lc_1	Lc_2	Lg	N	P	R_1	R_2	Rd	v	Y_1	Y_2
cl_1	50.0 240				53.3 122	47.3 240	48.3 240	47.6 63	47.5 61	50.0 240	46.0 74	16.7 240	45.8 240	55.5 63	56.1 240	59.3 86	
cr					55.9 84	41.2 245	47.4 240	54.2 24	6.6 61	50.9 240	64.0 75	49.6 240	46.0 240	29.2 24	50.8 240	53.5 86	
cn					51.3 119	47.0 181	46.7 45		45.6 58	56.7 90	48.3 197	46.2 211	50.3 190			52.6 58	47.4 211
cu						47.3 74	50.0 74	55.0 131		60.0 74	46.5 43	51.4 74					
fg					50.9 114	49.7 171	42.7 75		43.9 58	55.6 90	50.8 197	48.7 197	48.7 190			61.4 58	45.2 197
H_2					41.6 113	53.2 80	10.3 136	52.2 138	57.1 80	49.3 69	48.6 144	52.5 80	50.8 63	41.7 84	47.1 138	54.7 64	
L						49.6 240		45.5 80	53.9 240	53.6 69	44.6 240	51.9 240		50.8 240	55.0 80	46.4 183	
Lc_1							51.9 80	44.4 608	46.1 138	49.6 240	49.6 240			50.9 240	56.6 80	32.0 75	
Lc_2								49.3 73		56.1 82			52.4 63		50.7 73		
Lg									45.5 80	47.7 69	53.2 80	50.0 80		52.5 61	55.8 138		
N										56.9 69	52.2 240	49.6 240		52.6 240	59.2 80	50.7 138	
P											46.4 69	63.8 69		41.3 75	53.6 69	51.8 197	
R_1												50.2 240		47.1 240	51.9 108	51.8 214	
R_2														55.2 240	51.9 108	20.0 190	
Rd																	
v															61.6 86		
Y_1																55.6 190	

FIGURE 1.—Linkage relations in Upland cotton. The results of testing 18 mutant loci are presented based on 110 of the possible 153 paired combinations. The upper figure in each cell is the percentage recombination obtained in testcrosses; if it deviates from independence at the 5% level of significance the figure is underlined. The lower figure in each cell is the family size.

Let n be the number of plants in a backcross family and r be the number of recombinations. Deviation from independence is then calculated as a χ^2 value,

$$\chi^2 = [(n - r) - r]^2/n$$

TABLE 2

Maximum percent recombination which is distinguished from independence in backcross segregations, for various sizes of family and with two levels of precision ($P = .05$ and $P = .01$)

Family size	Percent recombination	
	$P = .05$	$P = .01$
10	19.01	9.27
20	28.09	21.20
30	32.11	26.48
40	34.51	29.64
50	36.14	31.78
60	37.35	33.37
70	38.29	34.61
80	39.04	35.60
90	39.67	36.42
100	40.20	37.12
200	43.07	40.89
500	45.62	44.24
1000	46.90	45.93

For a significant deviation from independence at the 5% level this χ^2 should be 3.841 or greater (from the appropriate tables). Thus $r = (n - \sqrt{3.841n})/2$. By substituting a series of values for n, the corresponding values of r can be determined. These are expressed in percent (100 r/n) in table 2. Similarly, at the 1% level, $r = (n - \sqrt{6.635n})/2$. It will be noted that except for the detection of very loose linkages, there is little advantage in increasing the family size above 100 plants.

EXPERIMENTAL RESULTS

In figure 1 significant departures from independence at the 5% level are underlined. Of the one hundred and ten combinations of loci tested for independence, seven were found to be linked. The complete backcross data for these seven combinations, supplemented by F_2 data when available, are shown in tables 3–9.

Linkage between R_1 and cl_1

The data shown in figure 1 indicate 16.7% crossovers between these two loci. As this linkage has been repeatedly observed the detailed analysis of the data will not be presented here.

Linkage between cr and L

Four backcross families, all in coupling gave homogeneous segregations (table 3a). Linkage was significant and a crossover value of 41.2% was obtained. These data are confirmed by the results of a single F_2 family, also in coupling, shown in table 3b. Despite a significant deficiency of crinkle segregates, linkage is significant and the crossover value of 41.1% is practically identical with the backcross estimate.

Linkage between cr and Lg

Data were available from one small backcross and one small F_2 family, both in coupling. Estimates of crossover values were in good agreement, giving 6.6% and 7.1% respectively (table 4).

TABLE 3

Linkage between cr *and* L

(a) Pooled data from 4 backcrosses

	L Cr	*L cr*	*l Cr*	*l cr*	Total	% c.o.
obs.	76	47	54	68	245	41.2
exp.	61.25	61.25	61.25	61.25	245	—

	D.F.	χ^2	*P*
L vs *l*	1	0.004	>0.90
Cr vs *cr*	1	0.918	0.50 – 0.30
Linkage	1	7.547	0.01 – 0.001
Total	3	8.469	

(b) Single F_2 family

	L Cr	*L cr*	*l Cr*	*l cr*	Total	% c.o.
obs.	400	80	131	50	661	41.1
exp.	372	124	124	41	661	—

	D.F.	χ^2	*P*
L vs *l*	1	2.002	0.20 – 0.10
Cr vs *cr*	1	10.026	0.01 – 0.001
Linkage	1	7.915	0.02 – 0.01
Total	3	19.943	

Linkage between Lc_1 and N

Four backcross families were examined, all in the coupling phase. Because two of the families involved only Upland (*hirsutum*) parents, while the others were interspecific first backcrosses (*barbadense* → *hirsutum*, and *tomentosum* → *hirsutum*) their segregations are presented separately in table 5. The segregations in the four families were homogeneous (χ^2 for 9 degrees of freedom = 3.61; $P = 0.95 - 0.90$) and their combined data showed significant evidence of linkage. A crossover estimate of 44.4% was obtained. There was a slight excess of Lc_1 segregates which was not significant in any of the individual families, though detectable in the combined data.

Linkage between Lc_1 and yg_2

Plants homozygous for yellow-green ($yg_2\ yg_2$) are very late in flowering, and as a result an appreciable number fail to mature seed in a normal growing season. This introduces an error in testing for linkage between yg_2 and the seed-hair character, Lc_1. One backcross and one F_2 family (both in coupling) were examined (tables 6a and 6b). It will be noted that in both families 14%–18% of the $yg_2\ yg_2$ plants failed

TABLE 4

Linkage between cr *and* Lg

(a) Backcross

	Lg Cr	*Lg cr*	*lg Cr*	*lg cr*	Total	% c.o.
obs.	25	0	4	32	61	6.6
exp.	15.25	15.25	15.25	15.25	61	—

	D.F.	χ^2	*P*
Lg vs *lg*	1	1.984	0.20 – 0.10
Cr vs *cr*	1	0.148	0.70
Linkage	1	46.049	<0.001
Total	3	48.181	

(b) F_2

	Lg Cr	*Lg cr*	*lg Cr*	*lg cr*	Total	% c.o.
obs.	111	5	4	24	144	7.1
exp.	81	27	27	9	144	—

	D.F.	χ^2	*P*
Lg vs lg	1	2.370	0.20 – 0.10
Cr vs *cr*	1	1.815	0.20 – 0.10
Linkage	1	69.444	<.001
Total	3	73.629	

TABLE 5

Linkage between Lc_1 *and* N

Family	Lc_1 *N*	Lc_1 *n*	lc_1 *N*	lc_1 *n*	Total	% c.o.
intra-*hirsutum* b.c.	19	18	15	25	77	
intra-*hirsutum* b.c.	71	56	48	60	235	
(*hirs.* × *barb.*) × *hirs.*	49	36	27	36	148	
(*hirs.* × *toment.*) × *hirs.*	43	38	32	35	148	
Combined data obs.	182	148	122	156	608	44.4
exp.	152	152	152	152	608	—

	D.F.	χ^2	*P*
Lc_1 vs lc_1	1	4.447	0.05 – 0.02
N vs *n*	1	0.000	—
Linkage	1	7.605	0.01 – 0.001
Total	3	12.052	

TABLE 6

Linkage between Lc_1 *and* yg_2

(a) Backcross

	$Lc_1\ Yg_2$	$Lc_1\ yg_2$	$lc_1\ Yg_2$	$lc_1\ yg_2$	Total	Unscored for Lc_1	
						Yg_2	yg_2
obs.	30	13	11	21	75	2	13
exp.	18.75	18.75	18.75	18.75	75	—	—

	D.F.	χ^2	*P*
Lc_1 vs lc_1	1	1.613	0.30 – 0.20
Yg_2 vs yg_2	1	0.653	0.80 – 0.20
Linkage	1	9.720	0.01 – 0.001
Total	3	11.986	

% crossover = 32.0.

(b) F_2

	$Lc_1\ Yg_2$	$Lc_1\ yg_2$	$lc_1\ Yg_2$	$lc_1\ yg_2$	Total	Unscored for Lc_1	
						Yg_2	yg_2
obs.	70	9	11	4	94	1	22
exp.	52	18	18	6	94	—	—

	D.F.	χ^2	*P*
Lc_1 vs lc_1	1	4.099	0.05 – 0.02
Yg_2 vs yg_2	1	6.255	0.02 – 0.01
Linkage	1	2.501	0.20 – 0.10
Total	3	12.855	

% crossover = 36.0.

to produce seeds. Estimates based on the remaining plants give crossover values of 32.0% and 36.0% for backcross and F_2 respectively. These values may be overestimates. Both in the backcross and in the F_2 the recombination classes, $Lc_1\ yg_2$ and $lc_1\ Yg_2$, are approximately equal while the noncrossover class $lc_1\ yg_2$ is deficient. This suggests that the majority of the yg_2 plants unscored for lint color might be of the constitution $lc_1\ yg_2$, in which case the crossover estimates obtained might be 10%–15% in excess of their true values. Independent evidence that the overestimate is not as serious as this will be presented later.

Linkage between R_2 *and* yg_2

Most of the $yg_2\ yg_2$ types produced flowers, so that the difficulties mentioned in the previous test were not encountered. Results from single backcross and F_2 families,

TABLE 7

Linkage between R_2 *and* yg_2

(a) Backcross

	$R_2\ Yg_2$	$R_2\ yg_2$	$r_2\ Yg_2$	$r_2\ yg_2$	Total	% c.o.
obs.	77	14	24	75	190	20.0
exp.	47.5	47.5	47.5	47.5	190	—

	D.F.	χ^2	*P*
R_2 vs r_2	1	0.337	0.70 – 0.50
Yg_2 vs yg_2	1	0.758	0.50 – 0.30
Linkage	1	68.400	<0.001
Total	3	69.495	

(b) F_2

	$R_2\ Yg_2$	$R_2\ yg_2$	$r_2\ Yg_2$	$r_2\ yg_2$	Total	% c.o.
obs.	68	11	13	21	113	22.3
exp.	64	21	21	7	113	—

	D.F.	χ^2	*P*
R_2 vs r_2	1	1.560	0.30 – 0.20
Yg_2 vs yg_2	1	0.664	0.50 – 0.30
Linkage	1	33.653	<0.001
Total	3	35.877	

both in coupling, are shown in table 7. The single gene ratios did not deviate significantly from expectation and the crossover estimates of 20.0% and 22.3% are in reasonably good agreement.

Linkage between H_2 and Lc_2

Through the courtesy of DR. J. O. WARE two different brown lint stocks, "Higginbotham Brown" and "Brymer Brown", both known to be independent of the Lc_1 locus, were made available to the author. Both types have light brown lint, Brymer being paler than Higginbotham. A third light brown lint type, similar phenotypically to Brymer, and transferred from *tomentosum*, was also tested. All three genes were found to be linked with Pilose (H_2). Presumably they are allelic, though it is possible that the Brymer and *tomentosum* genes are identical. The results of three backcrosses are presented in table 8a. The backcrosses involving Higginbotham and Brymer were in repulsion, that involving the *tomentosum* gene in coupling. The families were small and inspection suggests that there were less crossovers in the *tomentosum* backcross. However, contingency tests showed no significant departure from homogeneity,

TABLE 8

Linkage between H_2 *and* Lc_2

(a) Backcrosses involving:

	H_2 Lc_2	H_2 lc_2	h_2 Lc_2	h_2 lc_2	Total
(I) Higginbotham	3	13	17	1	34
(II) Brymer	1	13	19	6	39
(III) *tomentosum*	29	3	0	31	63

Joint estimate of % crossover = 10.3.

	D.F.	χ^2	*P*
H_2 vs h_2	1	1.059	0.50 – 0.30
Lc_2 vs lc_2	1	0.029	0.90 – 0.80
Linkage	1	85.765	<0.001
Total	3	86.853	

(b) F_2 (involving lint color gene from *tomentosum*)

	H_2 Lc_2	H_2 lc_2	h_2 Lc_2	h_2 lc_2	Total
obs.	34	3	5	11	53
exp.	30	10	10	3	53

	D.F	χ^2	*P*
H_2 vs h_2	1	0.761	0.50 – 0.30
Lc_2 vs lc_2	1	0.057	0.90 – 0.80
Linkage	1	24.908	<0.001
Total	3	25.726	

% crossover = 15.2.

neither in respect of the single gene segregations nor in the linkage component. From the combined families a joint estimate of 10.3% crossovers was obtained.

A single F_2 segregation (actually from a selfed brown lint segregate from a second backcross of *tomentosum* to *hirsutum*) was also examined (table 8b). The segregation is in coupling. An estimate of 15.2% crossovers was obtained which does not agree too well with the backcross estimate.

Double crossovers

The seven linked pairs of loci (fig. 1) include one four point and one three point linkage group. These are:—

N	Lc_1	yg_2	R_2
44.4	32.0	20.0	

L	*cr*	*Lg*
41.2	6.6	

Unfortunately only three groups of the families available were segregating concurrently for more than two pairs of loci belonging to the same linkage group. These all involved the four point linkage N-Lc_1-yg_2-R_2. The map intervals are rather large, N being very loosely linked with Lc_1, and the linkage between Lc_1 and R_2 only detectable by segregation in the intervening locus, yg_2. Also the total map distance has a minimum value of 96.4, so that no interference would be expected. The results from three groups of backcross families, each testing recombinations from 3-point linkages, are summarized in table 9. Interference is measured as a departure from independent crossing over in adjacent regions, using a form of contingency test for which I am indebted to DR. R. C. LEWONTIN. In any 3-point linkage, A-B-C, let p_1 and p_2 be the chances of a crossover occurring in the A-B and B-C interval, respectively. If

TABLE 9

Double crossovers in the N — Lc_1 — yg_2 — R_2 *region with appropriate tests for independence. In the contingency tests,* p_1 *and* p_2 *represent the chances of obtaining a crossover in the right and left map interval respectively. For further explanation see text*

Backcross data		Contingency test for independence*			
			p_1	$(1 - p_1)$	Total
(a) $N - Lc_1 - R_2$ (Map distance = 96.4)					
Noncrossovers	87	p_2	64	86	150
Doubles	64	$(1 - p_2)$	68	87	155
Singles ($N - Lc_1$)	68				
Singles ($Lc_1 - R_2$)	86	Total	132	173	305
Total	305	χ^2 (1) = 0.045; P = 0.90 — 0.80			
(b) $N - Lc_1 - yg_2$ (Map distance = 76.4)					
Noncrossovers	11	p_2	5.5	4.5	10
Doubles	6	$(1 - p_2)$	9.5	10.5	20
Singles (N — Lc_1)	9				
Singles ($Lc_1 - yg_2$)	4	Total	15.0	15.0	30
Total	30	χ^2 (1) = 0.150; P = 0.70 — 0.50			
(c) $Lc_1 - yg_2 - R_2$ (Map distance = 52.0)					
Noncrossovers	29	p_2	1.5	15.5	17.0
Doubles	1	$(1 - p_2)$	20.5	29.5	50.0
Singles ($Lc_1 - yg_2$)	21				
Singles ($yg_2 - R_2$)	16	Total	22.0	45.0	67.0
Total	67	χ^2 (1) = 5.956; P = 0.02 — 0.01			

* Classes adjusted by Yates' correction.

TABLE 10

Test for random distribution of 18 mutant loci over the total complement of 26 chromosomes. The expected frequencies are based on a Poisson distribution

No. of loci per chromosome	0	1	2	3	4	Total	Mean	Variance
observed	15	7	2	1	1	26	0.692	1.102
expected	13.0	9.0	3.1	0.9		26	—	—

χ^2 (2) = 2.486; P = .30 − .20.

there is no interference (p_1 independent of p_2) the expected composition of the resulting gametic classes will be:—

Noncrossovers	$(1 - p_1)(1 - p_2)$
Doubles	$p_1 p_2$
Singles (A-B)	$p_1 (1 - p_2)$
Singles (B-C)	$p_2 (1 - p_1)$
Total	1

Hence departures from expectation are indicative of interference and can be measured with a 2 × 2 contingency test in the usual way (table 9).

The data show no evidence of interference in the map intervals, *N*-Lc_1 and Lc_1-R_2 (table 9a) nor in the map intervals, *N*-Lc_1 and Lc_1-yg_2 (table 9b). However there is a significant departure from independence in the map intervals, Lc_1-yg_2 and yg_2-R_2 (table 9c). The family size is small, nevertheless the recovery of only one double crossover in a map distance of 52 units is surprisingly low (coincidence = 0.18).

As mentioned earlier, there is reason to suspect that the map distance between Lc_1 and yg_2 may be overestimated. However, the fact that there is no detectable linkage between the Lc_1 and R_2 loci means that they cannot be separated by much less than 50 crossover units. Since the distance between yg_2 and R_2 is of the order of 20–22 units, the minimal distance between Lc_1 and yg_2 should be 50 minus 22, i.e. 28 units as compared with the probable overestimate of 32% in table 6a.

DISCUSSION

Distribution of mutant loci

The eighteen mutants tested fall into a maximum of eleven linkage groups:—

(1) *N*-Lc_1-yg_2-R_2
(2) *L*-*cr*-*Lg*
(3) R_1-cl_1
(4) H_2-Lc_2

with seven other loci (*cn*, *cu*, *fg*, *P*, *Rd*, *v* and V_1) independent of each other and of the other four linkage groups. The remaining fifteen chromosomes of the total set of twenty-six are thus unmarked. If the eighteen mutants were distributed at random over the genome the frequencies of chromosomes carrying 0, 1, 2 etc. mutant loci should conform to a Poisson distribution. The actual and expected frequencies (table

10) do not differ significantly though there is a tendency for too many chromosomes to carry no mutant loci and for too many mutants to be concentrated in multiple linkage groups. In spite of its statistical nonsignificance there are four reasons for believing this tendency to be a real one:

(1) In a sample conforming to a Poisson distribution the mean and variance should have the same order of magnitude. The observed data in table 10 have a variance almost twice as large as the mean.

(2) The number of linked combinations recorded in figure 1 might be an underestimate. Owing to limitations of family size, loose linkages might escape detection.

(3) Only 110 out of the possible 153 combinations were tested (fig. 1). The combinations not tested might have revealed additional linkages.

(4) Two other mutant loci, not tested here, are known to belong to existing linkage groups. These are cl_2 in linkage group 1 and *ck* in linkage group 2.

All these factors suggest that the proportion of linked combinations is greater than has been detected and that as a consequence, the proportion of independent linkage groups should probably be smaller. In table 10 this should have the effect of increasing the frequencies of chromosomes with 2 and 3 loci at the expense of those with 1 locus and therefore of producing a wider departure from the Poisson distribution. On the whole it seems likely, though not proven, that the mutant loci are not randomly distributed but tend to be concentrated in certain chromosomes.

Since the *hirsutum* cottons are amphidiploids, a possible explanation of a nonrandom distribution of mutants might be a historical one, namely that one of the diploid parents had more mutable loci than the other. Evidence on this point is meager at present but so far does not favor this interpretation. Since HARLAND (1935) showed that R_2 was located in the A subgenome it follows that the other three genes in linkage group (1) are also located there. HUTCHINSON (unpublished) showed that a green lint gene occurring in the diploid D species, *G. armourianum*, was homologous with *Lg* in *hirsutum* and also linked with crinkle (*cr*). Later GREEN (1953) showed that the leaf-shape gene of another diploid D species, *G. thurberi*, was an allele of the *L* series in *hirsutum*. Clearly the genes in linkage group (2) are located in the D subgenome. Linkage group (3) is in the D subgenome (HARLAND 1937). Linkage group (4) is still unlocated. The unlinked loci, *P* and Y_1, are both in the A subgenome (STEPHENS 1954a, b). Two other loci, not tested here, have also been located; corky (*ck*) in the D subgenome (STEPHENS 1950) and short-branch (cl_2) in the A subgenome (SILOW 1946). Altogether, there are six loci definitely placed in the D subgenome and seven in the A subgenome. This suggests that a nonrandom occurrence of mutants would be associated with differences between specific chromosomes and not between chromosome sets.

The question of chiasma frequency

The somatic chromosomes of *G. hirsutum*, as illustrated by SIKKA *et al.* (1945) and WOUTERS (1948), tend to have median or submedian centromeres. In metaphase I of meiosis, ring bivalents are regularly formed, though a low proportion of rod bivalents also occurs (SKOVSTED 1934). According to MENZEL and BROWN (1945) the typical metaphase I bivalent in *hirsutum* has "one chiasma in each arm" each chiasma being

"usually terminal or nearly so". The conclusion therefore seems justified that *on the average* the *hirsutum* chromosome has one chiasma in each arm at metaphase I. Meiotic prophase stages in cotton are technically difficult to study, and as yet there is no reliable evidence to determine whether the low chiasma frequency at metaphase is the result of terminalization, or of a low initial frequency at pachytene. In Asiatic cottons, SKOVSTED (1933) observed a maximum of three chiasmata per bivalent between diplotene and metaphase, but the number of cells examined was not recorded and in any case his observation cannot be directly applied to the situation in other species of Gossypium. The question is important, because an initially low chiasma frequency at pachytene should limit the rate of genetic recombination in a hybrid population. It may account for certain discrepancies which are found when apparently identical genes are transferred by backcrossing to a "common" background (STEPHENS 1945).

The mapping of *hirsutum* chromosomes, here commenced, should make possible a critical examination of the initial chiasma frequency. If the hypothesis of one chiasma in each arm is valid, one could predict that *on the average* the total map distance for a *hirsutum* chromosome would be of the order of 100 crossover units (50 per arm) and that double crossovers within any arm should be rare or nonexistent. Interference should be a common phenomenon and tend to be independent of map distance. The linkage groups at present available are quite inadequate to test the hypothesis, but it is interesting that the N-Lc_1-yg_2-R_2 linkage group seems to be in agreement with it. Here the map distance is 96.4, crossing over to the left of Lc_1 is independent of crossing over to the right of this locus, and there is practically complete interference between the intervals on either side of yg_2. The interference cannot be attributed to proximity of two short intervals since the total distance between Lc_1 and R_2 must be of the order of 50 units or greater. The simplest interpretation at present is that a chiasma regularly formed in the left arm usually occurs between N and the centromere and that a chiasma regularly formed in the right arm falls either between the centromere and yg_2 or between yg_2 and the end of the right arm. Only rarely is a second chiasma formed in the right arm. On this interpretation, the distance of about 50 units between Lc_1 and R_2 would indicate that these loci are situated near the centromere and near the end of the right arm respectively (fig. 2). It should be fairly simple to test the validity of this interpretation as new loci are added to

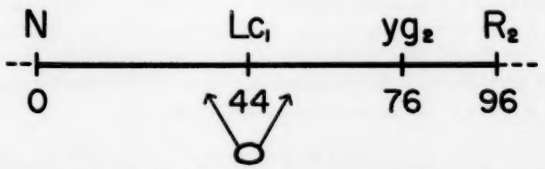

FIGURE 2.—Provisional genetic map of linkage group 1 in Upland cotton. The open circle indicates the probable position of the centromere, based on the assumption that on the average only one chiasma is formed per arm, and that as a consequence the total map distance is approximately 100 units.

For further explanation see text.

the linkage group. If it is substantially correct, few loci should be discovered to the left of *N* or to the right of R_2 and all new map intervals associated with placement of additional loci should exhibit interference with either or both of their adjacent map intervals and irrespective of their map distances.

The location of short-branch, cl_2, is of particular interest in this connection. It is known to be approximately sixteen map units away from R_2 (SILOW 1946). On the interpretation presented here it would be expected that cl_2 would be located to the right of yg_2 and closely linked with it.

SUMMARY

Eighteen loci in Upland cotton have been screened for linkages, 110 of the possible 153 paired combinations of loci being tested in appropriate backcrosses. Seven linked combinations were obtained which could be arranged in 4 linkage groups as follows (testcross data only):

(1) *N* — 44.4 — Lc_1 — 32 — yg_2 — 20 — R_2 (A subgenome)

(2) *L* — 41.2 — *cr* — 6.6 — *Lg* (D subgenome)

(3) R_1 — 16.7 — cl_1 (D subgenome)

(4) H_2 — 10.3 — Lc_2 unknown

The correct order of cl_2 in (1) and *ck* in (2) is not yet established. There is an apparent tendency for the mutant loci to "cluster" in specific chromosomes, though the numbers are not large enough to demonstrate a significant departure from a random (Poisson) distribution. However, of the 18 mutants tested 7 can be shown to be located in the A subgenome and 6 in the D subgenome, so that any departure from random distribution would be associated with specific chromosomes rather than specific subgenomes. The metaphase I chromosome in Upland cottons has typically a median or submedian centromere and one chiasma in each arm. If this chiasma frequency represents a low initial frequency rather than the result of terminalization, one could predict that genetic map distances should approximate to 100 units (50 per arm) and that double crossovers should be rare or absent in any one arm, irrespective of map distance. It is pointed out that the information available from linkage group 1 is not at variance with this prediction.

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A COMPARATIVE STUDY OF CROSSING OVER IN ATTACHED-X CHROMOSOMES OF DROSOPHILA MELANOGASTER[1]

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THE work of L. V. MORGAN (1925) demonstrated that crossing over in attached-X chromosomes of *Drosophila melanogaster* could be systematically studied if one could obtain attached-X chromosomes which were properly marked. From her experiments with these attached chromosomes, MORGAN concluded that at least sometimes crossing over occurred when four chromatids were present. In this same paper MORGAN indicated that by observing the various kinds of attached-X offspring of an attached-X mother, one could determine the genotype of the heterozygous mother.

The first experiment designed for the systematic study of crossing over in attached-X chromosomes was performed by ANDERSON (1925). This piece of work succeeded in demonstrating the following pertinent points: (1) Crossing over in attached-X chromosomes appeared to proceed normally and regularly in spite of the fact that the two chromosomes were obliged to behave as a unit. (2) Single exchanges between non-sister chromatids occurred at random. (3) Crossing over regularly occurred at the four strand stage and only two of the four chromatids crossed over at any one level. (4) The homozygosis frequency of the recessive allele carried by the X-chromosomes decreased in a regular fashion as the position of the alleles approached one end of the chromosome, at which end, it was inferred, the centromere must be located. In addition to the above mentioned points, ANDERSON demonstrated in detail the method of progeny testing whereby the genotype of an attached-X female could be determined.

EMERSON and BEADLE (1933) published the results of an experiment the purpose of which "was to obtain more accurate data concerning the relative frequencies of different crossover types." These data supported ANDERSON'S conclusions and in addition made possible a comparison of the frequency of 2-strand and 4-strand double crossover types. In order to determine the ratio of these types, the investigators incorporated the data of ANDERSON and a large block of data from STURTEVANT (1931). The combined data demonstrated a 1:1 ratio between 2-strand and 4-strand double exchanges for those cases in which the first detectable exchange nearest the spindle fiber is non-reciprocal; *i.e.* the exchange occurs between chromatids which are destined to be attached to different centromeres at the second meiotic division. This 1:1 ratio indicated that the first exchange in no way determined what type of second exchange would occur. In other words, there was an absence of chromatid interference.

BEADLE and EMERSON (1935) then performed another experiment using attached-X

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chromosomes which were marked from scute to carnation. All previous conclusions were confirmed. When allowance was made for the possible presence of lethal mutations, it was found that the 2-strand and 4-strand double exchanges occurred in equal frequency. Furthermore, data were obtained concerning the occurrence of 2-strand and 3-strand double exchanges in those cases where the first detectable exchange was reciprocal. A reciprocal first exchange occurs between two chromatids which are destined to be attached to the same centromere during the second meiotic division. In the absence of chromatid interference these 2-strand and 3-strand doubles should occur in equal frequency. BEADLE and EMERSON found this to be true.

Up to this time, all the work performed with attached-X chromosomes tended to indicate that exchanges occurred at random between non-sister chromatids, and that when multiple exchanges occurred, there was an absence of chromatid interference. However, BONNIER and NORDENSKIÖLD (1937) claimed to have demonstrated an excess of non-reciprocal single exchanges and in the case of double exchanges they believed that there was an excess of 4-strand doubles. Thus they claimed to have demonstrated chromatid interference. The work of BONNIER and NORDENSKIÖLD has not been repeated, and no further significant studies have been performed which utilize the advantages made available by the use of attached-X chromosomes.

It should be noted at this point that in the investigation concerned with crossing over in attached-X chromosomes, all the investigators except BONNIER and NORDENSKIÖLD performed their experiments by utilizing chromosomes which were marked in essentially the same manner. That is to say, the recessive markers were carried by the chromosomes in an alternate fashion at succeeding loci. Many of the data were obtained from flies with various recessive phenotypes, and precautions had to be taken to prevent the introduction of a bias caused by viability differences. BONNIER and NORDENSKIÖLD varied the scheme by placing all the mutant alleles on one chromosome and all the wild alleles on the other. If one performs the experiment in this way, it is possible to base one's conclusions on data derived from progeny tests of flies which are all of a wild type phenotype.

The decision was made to perform another experiment utilizing the method of BONNIER and NORDENSKIÖLD in an attempt to affirm or deny the presence of chromatid interference. It was soon discovered that an autosomal inversion could be inserted into the properly marked attached-X females. Therefore, the original study was expanded so as to be a comparative study of crossing over in attached-X chromosomes in the presence of an autosomal inversion and in the absence of one. DREW SCHWARTZ (1953) has uncovered new evidence which indicates that crossing over can occur between sister strands, and it seems especially desirable at this time to re-examine or add new data to the existing evidence upon which much of our knowledge of crossing over is based.

SYNTHESIS OF ATTACHED-X FEMALES

In order to synthesize attached-X females which had all the recessive mutant alleles located on one chromosome, a marked chromosome had to be inserted into the attached-X complex. The method used in this study was to make attached-X

chromosomes by taking advantage of crossovers which occur between a duplication carried by an X-chromosome, and the homologous segment of a normal chromosome. The X-chromosome bearing the duplication B^s (Bar of STONE) was first synthesized by MULLER (1936). Females heterozygous for the duplication are viable and fertile. When a crossover occurs between the duplication and the homologous segment in the normal chromosome, an attached-X chromosome is formed. Furthermore, when the crossover occurs, the resulting attached-X females are free of the B^s and hence have a wildtype phenotype. By making the proper crosses, one can pick out newly made attached-X females by selecting those females that lack the Bar phenotype.

The required exchange apparently occurs very infrequently unless some stimulus to crossing over is applied. The third chromosome Dichaete inversion, *In(3)DcxF*, was introduced into females which were already heterozygous for the duplicated X-chromosome, and under the stimulus of this inversion, attached-X females began to appear at the rate of approximately one in 900.

The above rate of production of the attached-X type of females was sufficient for the purpose of this experiment. Females were produced which were heterozygous for the duplicated X-chromosome and another X-chromosome bearing all the recessive mutant alleles to be used as the markers for this experiment. The occurrence of the type of crossover which makes an attached-X would then simultaneously make the complex heterozygous for the recessive alleles. All the recessive alleles would be located on one chromosome unless an exchange in the duplicated region was followed by another exchange distal to this region of duplication. In that event, the markers just to the left of the distal exchange would be transferred to the other X-chromosome or completely eliminated from the attached-X complex. As long as double exchanges of this type did not occur too frequently, it was no cause for alarm because the progeny testing which followed would soon betray the actual genotype of the resulting attached-X female.

For reasons which will be discussed later, only that portion of the chromosome within about 40 units of the spindle fiber attachment was marked for study. This portion of the chromosome was well marked from the locus of pentagon to the locus of forked. The markers which were used, their symbols, and their localization according to BRIDGES and BREHME (1944) are as follows: (1) pentagon^3, *ptg*^{3}, 23.2 (2) vermilion, *v*, 33.0 (3) miniature, *m*, 36.1 (4) garnet^2, *g*^{2}, 44.4 (5) scalloped, *sd*, 51.5 (6) forked, *f*, 56.7. No two neighboring loci are more than 9.8 map units apart. With loci so closely spaced there should not be an appreciable amount of undetected double exchanges occurring between the neighboring loci. Females which are homozygous for all six mutant alleles are sterile, but hemizygous males are fertile. No sterility trouble was encountered, since the males were used to introduce the marked chromosome into the situation in which an exchange between it and the duplicated X-chromosome would produce an attached-X female.

Since the Dichaete inversion is present, it is expected that half of the synthesized attached-X females will carry the inversion and the other half will be free of it. By utilizing both types of attached-X females, it was possible to make a comparative study of crossing over. It should be pointed out that the dominant phenotype of the Dichaete inversion does not interfere with the recognition of phenotypes resulting from homozygosity of the sex-linked recessive alleles.

PRODUCTION OF ATTACHED-X PROGENY

When the technique outlined above yielded an attached-X female, the fly was transferred to a one ounce creamer which was approximately half full of food, well yeasted, and covered with a layer of wheat germ. The female was allowed to lay for a period of six days. The fly was then transferred to a fresh creamer and allowed to lay for four days more. Another transfer was followed by another laying period of four days, and finally the fly was transferred once again and allowed to lay until death. Attached-X females that were also phenotypically Dichaete required some special handling. Since the wings of such a female are always wide spread, the flies frequently became stuck to the medium or to the sides of the creamer. Therefore, half of each wing was removed, as a result of which they frequently survived long enough to yield an appreciable number of offspring. The purpose of the wheat germ was two-fold; it served both to fortify the medium with B vitamins and to take up the excess water.

The progeny furnished the data which revealed the process of crossing over as it had occurred in the parental female. It would have been possible to use the progeny of original attached-X females as the source of additional progeny and repeat this procedure generation after generation until the study was completed. However, this method may lead to the accumulation of recessive lethals in the attached-X chromosomes and thereby eliminate certain crossover classes of progeny. For this reason, new attached-X's were synthesized afresh, and in general, but not always, the data were derived from their immediate progeny.

COLLECTION OF PROGENY FROM PARENTAL FEMALES

Every parental female which is heterozygous for the marker genes listed above will give rise to a group of progeny which is phenotypically variable. Some flies will be wild type in appearance and may be homozygous or heterozygous for the wild type alleles. Some will be homozygous for one recessive allele and consequently show that characteristic phenotype. Others will be homozygous for a combination of recessive alleles and consequently will show a different recessive phenotype. The offspring of the parental females were collected as they emerged. If any one of the attached-X progeny had a recessive phenotype, this fact was recorded and the fly was discarded. If the emerging fly had a wild type phenotype, this fact was recorded also, and then the fly was placed in a fresh creamer with Muller-5 males in order to progeny test it. By collecting progeny from such a female it is possible to determine its exact genotype and to decide what sort of crossover, if any, had taken place.

RECIPROCAL VERSUS NON-RECIPROCAL EXCHANGES

Reciprocal and non-reciprocal exchanges lead to derivatives which are specific for the type of exchange. They may be identified upon progeny testing the offspring of such a female. In figure 1 note that the reciprocal exchange yields either a heterozygous crossover attached-X (1) or a heterozygous non-crossover attached-X like the mother (2). A non-reciprocal exchange yields two attached-X's which are homozygous beyond the point of exchange. In one case the mutant alleles become homozygous (3) and in the other case it is the wild type alleles which become homozygous

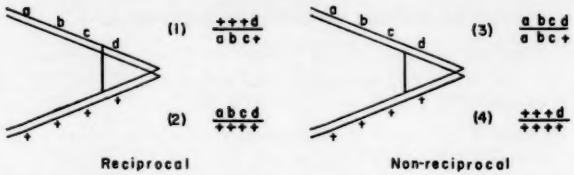

FIGURE 1.—The possible attached-X products of a reciprocal and a non-reciprocal exchange are shown above. The products of a reciprocal exchange are heterozygous to the left of the exchange point while the products of a non-reciprocal exchange are homozygous.

(4). If single exchanges occur, and these at random between non-sister chromatids, and if only wild type flies are progeny tested, then the two types +++d/abc+ and +++d/++++ should be found with equal frequency.

The results obtained from progeny testing 1638 wild type offspring are listed in table 1. For each region between two marker genes, the frequency of reciprocal and non-reciprocal exchanges was totaled and listed in table 2. It should be emphasized that in those cases where double exchanges occurred, only the rightmost exchange was noted because it is not always possible to identify the type of crossover which has occurred at the second point of exchange. The occurrence of double exchanges introduces a bias into the expected 1:1 ratio between reciprocal and non-reciprocal exchanges; the act of listing only the right-most exchanges does not alleviate the bias. However, the extent of the bias is proportional to the frequency of double exchanges, and the bias will be in the direction of an excess of non-reciprocal exchanges. As will be shown later, double exchanges were not frequent enough to cause an appreciable deviation from the expected 1:1 ratio of reciprocal to non-reciprocal exchanges. This topic will be pursued further when double exchanges come under discussion.

It is obvious from an examination of table 2 that the agreement between observation and expectation is very good. The reciprocal and non-reciprocal exchanges occur in equal frequency. No comparison for the region from forked to the spindle fiber can be made because reciprocal exchanges in this region remain undetected.

Before proceeding further it might be well to stop and ask if the data from which these exchanges were obtained are homogeneous. It happens that the 1638 wild type offspring of table 1, and hence the total of 604 reciprocal and non-reciprocal exchanges, were obtained from a total of 16 parental females. In order to test for homogeneity, the observed numbers of reciprocal and non-reciprocal exchanges produced by each parental female were compared with an expected ratio of 1:1. Since the total exchanges were obtained from 16 parents, we have 16 separate chi-squares which can now be summed; the degrees of freedom also number 16.

The sum of the chi-squares equals 10.59 for 16 degrees of freedom. The chi-square value of the total 298 reciprocal: 306 non-reciprocal equals 0.11 for one degree of freedom. This corresponds to a P value between 0.7 and 0.8. This chi-square value with its one degree of freedom can be subtracted from the sum of 10.59 to yield a chi-square of 10.48 for 15 degrees of freedom which is a measure of heterogeneity or

TABLE 1

The genotypes of wild type females as revealed by progeny tests

Attached-X genotype	+/+*	D/+†	Attached-X genotype	+/+*	D/+†	Attached-X genotype	+/+*	D/+†
plg v m g sd f / + + + + + +	982	465	*plg* + + + + *f* / + + + + + +	1	1	*plg v* + + + + / + + + + *sd f*		1
+ + + + + + / + + + + + +	42	28	*plg* + + + + + / + + + + + *f*	3		*plg v m* + *sd f* / + + + *g* + +	1	
plg + + + + + / + *v m g sd f*	87	60	*plg v* + + + *f* / + + *m g sd* +	1	1	*plg v m* + + + / + + + + *sd f*		2
+ *v m g sd f* / + + + + + +	103	43	+ + + + + *f* / + + *m g sd* +	1	2	*plg* + + *g sd f* / + *v m* + + +		1
plg v + + + + / + + *m g sd f*	42	12	*plg* + + + + *f* / + + + + + +	1		+ + + *g sd f* / + *v m* + + +	2	1
+ + *m g sd f* / + + + + + +	37	15	*plg v* + + + + / + + + + + *f*		2	*plg* + + *g sd f* / + + + + + +		4
plg v m + + + / + + + *g sd f*	63	45	*plg v m* + + + / + + + + + *f*	1		*plg* + + + + + / + + + *g sd f*	4	2
+ + + *g sd f* / + + + + + +	56	55	*plg* + + + *sd f* / + *v m g* + +	1	2	*plg* + + + + + / + + *m g sd f*	1	
plg v m g + + / + + + + *sd f*	48	30	+ + + + *sd f* / + *v m g* + +	1	1	*plg* + + + + + / + + + + + +	8	7
+ + + + *sd f* / + + + + + +	54	32	*plg* + + + *sd f* / + + + + + +	3	2	*plg v* + + + + / + + + + + +		2
plg v m g sd + / + + + + + *f*	45	23	*plg* + + + + + / + + + + *sd f*	3	1	*plg v m* + + + / + + + + + +		5
+ + + + + *f* / + + + + + +	37	19	*plg v* + + *sd f* / + + *m g* + +		1	*plg v m g* + + / + + + + + +	1	
plg + + + + *f* / + *v m g sd* +	4	1	+ + + + *sd f* / + + *m g* + +		1	+ + *m* + + + / + + + + + + +	1	
+ + + + + *f* / + *v m g sd* +	2		*plg v* + + *sd f* / + + + + + +	2		Total	1638	867

* *In(3)DcxF* not present.
† *In(3)DcxF* present.

TABLE 2

The regional frequencies of reciprocal and non-reciprocal exchanges

		pig-v	*v-m*	*m-g*	*g-sd*	*sd-f*	*f-sp.f.*	Total
In(3)DcxF absent	R*	87	42	65	51	53	52	298
	NR†	103	38	60	62	43		306
	Total	190	80	125	113	96		604
	χ^2	1.35	0.20	0.29	1.07	1.47		0.11
In(3)DcxF present	R*	60	12	47	35	27	42	181
	NR†	43	15	61	38	22		179
	Total	103	27	108	73	49		360
	χ^2	2.81	0.33	1.82	0.12	0.51		0.01

* R = Reciprocal exchange.

† NR = Non-reciprocal exchange.

disagreement among the groups. The *P* value is again between 0.7 and 0.8 and the data can be considered homogeneous.

If we add an autosomal inversion to the parental attached-X females, what effect will this have on the crossing over? Will the addition of an autosomal inversion alter the comparative frequencies of the two types of exchanges, or will they still occur in the expected ratio of 1:1? Table 2 indicates that there is not a significant deviation from the expected 1:1 ratio. The actual frequencies of exchanges between any two loci are altered by the addition of an inversion so that, in general, the amount of crossing over increases (table 6), but no modification in the ratio of reciprocal to non-reciprocal exchanges has occurred.

Since the exchanges discussed above were found among the offspring of 19 different parental females, the question again arises as to whether or not the separate blocks of data are homogeneous. For the total of 181 reciprocal to 179 non-reciprocals the heterogeneity chi-square has a value of 20.52 for 15 degrees of freedom. The *P* value lies between 0.20 and 0.10 and therefore is not significant.

DOUBLE EXCHANGES

With a source of attached-X females marked as they are in this experiment, it is possible to obtain directly the relative frequencies with which certain recognizable double exchanges occur. The result can then be compared with expectation. Figure 2 has been constructed in order to demonstrate the derivation of ratios that should be found to exist if double exchanges occur at random. This figure is patterned after one constructed by EMERSON and BEADLE (1933).

It should be emphasized that the function of figure 2 is to demonstrate the various double crossover types. In the diagram the exchanges have occurred between the markers *c-d* and *a-b*. The double exchanges could occur differently within the marked section of the chromosome, for example, between *b-c* and *a-b*. The configuration of the derivatives of the double exchanges would be quite similar to those shown in figure 2 except that a smaller piece of the chromosome has been shifted about by the double exchange.

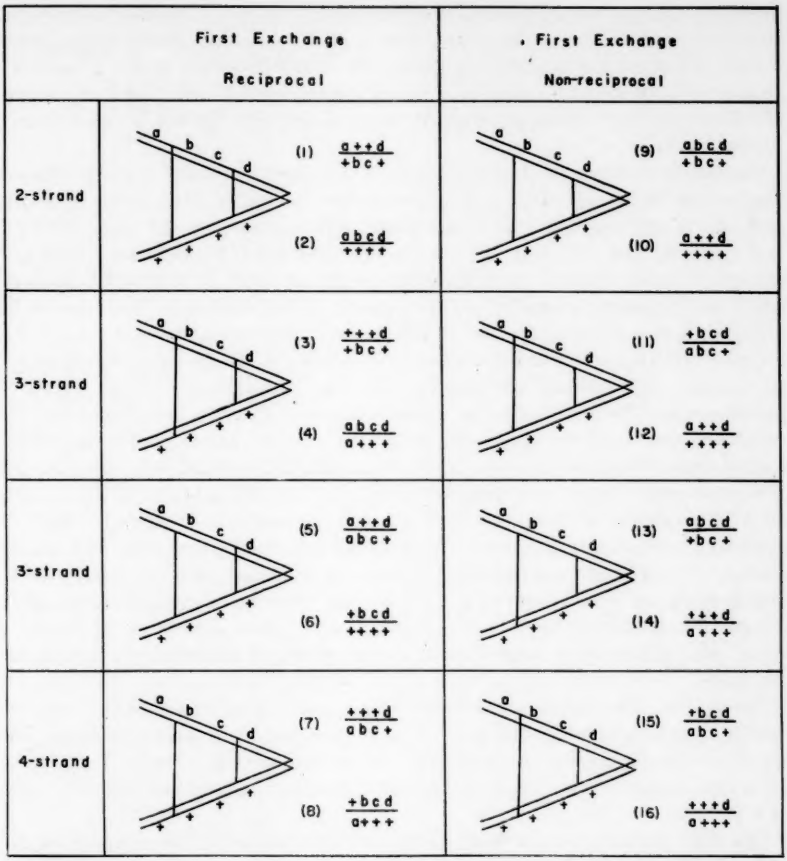

FIGURE 2.—The crossover types recovered from the eight different double exchanges which can occur between the non-sister chromatids of attached-X chromosomes.

The rightmost exchange, that is, the exchange nearest the centromere, will be referred to as the first exchange. As noted previously, this exchange may be reciprocal or non-reciprocal with equal frequency. Once the first exchange is determined, the remaining exchange can occur in such a way as to form a 2-strand, 3-strand, or 4-strand double exchange within the tetrad. If double exchanges occur at random, 2-, 3-, and 4-strand double exchange tetrads should exist in a ratio of 1:2:1.

Note those cases in which the first exchange is reciprocal. From these tetrads listed in figure 2, it is possible to recognize certain derivatives as resulting from the occurrence of double crossing over. The types $a++d/+bc+$ (1) and $+++d/+bc+$ (3) are two such derivatives. All other attached-X derivatives from a reciprocal first

exchange resemble non-crossovers, single crossovers, or bear a recessive phenotype and hence would not be further tested. Therefore, if attached-X female progeny with a wild phenotype are selected and tested, the double crossover types (1) and (3) should be found to exist in a ratio of 1:1. Type (1), $a++d/+bc+$, is a derivative of a 2-strand double exchange; type (3), $+++d/+bc+$, is derived from a 3-strand double exchange.

Another ratio which can be tested is one which should be found to exist in those cases where the first exchange is of the non-reciprocal type. If this happens to be the case, the double exchanges which are recognizable as such are the types $a++d/++++$ (10) and (12), and $+++d/a+++$ (14) and (16); all other double exchanges in which the first exchange is non-reciprocal result in attached-X females which have a recessive phenotype. By progeny testing wild type flies, we should find again a 1:1 ratio between the two types $a++d/++++$ and $+++d/a+++$.

One other ratio can be tested in those cases where the first exchange is non-reciprocal. Figure 2 shows that numbers (9), (11), (13) and (15) all bear the recessive phenotype *bc*. This phenotype can appear every time a double exchange occurs in which the first exchange is non-reciprocal; the recessive phenotype of these flies enables one to recognize them as having been derived from a double exchange. If this phenotypic class is then progeny tested, the two genotypes $abcd/+bc+$ and $abc+/+bcd$ should be found in a ratio of 1:1 if doubles occur at random. Most of these doubles bearing a recessive phenotype were picked up in the course of progeny testing wild type flies; their phenotype indicated that they were the product of a double exchange. They cannot be treated on a par with the other double exchanges. Inasmuch as doubles do not occur frequently in such a short segment of the chromosome, this comparatively large class of doubles formed a valuable contribution to the study.

Among the wild type flies, one more ratio remains to be tested. If we compare double exchanges in which the first exchange is reciprocal to double exchanges in which the first exchange is non-reciprocal, we should observe a 1:2 ratio. Thus types (1) and (3) versus types (10), (12), (14), and (16) should be found to exist in a ratio of 1:2.

The data pertinent to the ratios we have been considering are summarized in tables 3 and 4. In table 3 will be found a summary of data in which double exchanges have resulted in females homozygous for one or more mutant alleles. These double exchanges are equivalent to the types (9), (11), (13), and (15) of figure 2. In all future discussion the identical types (9) and (13) which result in the first case from a 2-strand double exchange and in the second case from one of the 3-strand doubles, will be referred to as a type-1 double and symbolized as $abcd/+bc+$. The remaining identical types, (11) and (15), will be referred to as a type-2 double and symbolized as $abc+/+bcd$. When we are concerned with those doubles that have a wild phenotype, the same procedure will be followed. The identical types (10) and (12), which may result either from a 2-strand or a 3-strand double exchange, will be referred to as a type-1 double and symbolized as $a++d/++++$. Types (14) and (16), which are identical and can come about as the result of a 3-strand or 4-strand double exchange, will be symbolized as $+++d/a+++$ and referred to as a type-2 double.

TABLE 3

The frequencies of type-1 and type-2 double exchanges obtained from offspring homozygous for one or more mutant alleles

Phenotype of offspring	Genotype of parent	Type-1 double		Type-2 double	
		+/+*	D/+**	+/+*	D/+**
v m g	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	14	3	18	4
m g sd	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	5	1	4	2
"	$\frac{plg\ +\ +\ +\ +\ +}{+\ v\ m\ g\ sd\ f}$	0	—	1	—
"	$\frac{+\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	—	0	—	1
v m	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	13	4	9	2
"	$\frac{plg\ v\ m\ g\ +\ +}{+\ +\ +\ +\ +\ +}$	3	—	1	—
"	$\frac{plg\ v\ m\ g\ sd\ +}{+\ +\ +\ +\ +\ f}$	1	—	1	—
m g	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	3	0	4	1
g sd	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	7	3	5	2
"	$\frac{plg\ +\ +\ +\ +\ +}{+\ v\ m\ g\ sd\ f}$	—	1	—	1
"	$\frac{+\ +\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	0	—	1	—
v	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	4	3	1	0
"	$\frac{plg\ v\ m\ g\ sd\ +}{+\ +\ +\ +\ +\ f}$	—	2	—	0
"	$\frac{plg\ v\ m\ +\ +\ +}{+\ +\ +\ g\ sd\ f}$	—	0	—	1
m	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	2	0	0	1
g	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	3	2	1	1
"	$\frac{plg\ +\ +\ +\ +\ +}{+\ v\ m\ g\ sd\ f}$	1	—	0	—
sd	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +}$	0	4	1	0
"	$\frac{plg\ v\ +\ +\ +\ +}{+\ +\ m\ g\ sd\ f}$	1	—	0	—
	Total	56	23	47	17

* *In(3)DcxF* not present.
** *In(3)DcxF* present.

TABLE 4

A summary of the double exchanges obtained from attached-X females

	First exchange reciprocal		First exchange non-reciprocal					
			Type-1		Type-2		Type-1:Type-2	
	2-strand a++d/ +bc+	3-strand +++d/ +bc+	a++d/ ++++	abcd/ + bc+	a+++/ +++d	abc+/ +bcd		
In(3)Dcxf absent	7	6	7	56	12	47	63	59
In(3)DcxF present	6	5	7	23	8	17	30	25

Table 4 contains the sum of the data found in table 3 and table 1. In those cases where the first exchange is reciprocal, 2-strand type, $a++d/+bc+$, and the 3-strand type, $+++d/+bc+$, would be expected to appear in equal frequency; the observed values are 7 and 6. In those cases where the first exchange is non-reciprocal, the type-1 and the type-2 doubles should be found to exist in a ratio of 1:1. The observed frequencies are 7 and 12. To these 19 cases one is able to add the sum of the type-1 and type-2 doubles of table 3. This results in a total of 63 type-1 ($a++d/++++$ and $abcd/+bc+$) and 59 type-2 ($+++d/a+++$ and $abc+/+bcd$). The agreement with an expected 1:1 ratio is very good.

One ratio remains to be tested. By restricting ourselves to the wild type data obtained by progeny testing, we should find that double exchanges in which the first exchange is reciprocal should be just one half as frequent as double exchanges in which the first exchange is non-reciprocal. In other words, we expect a ratio of 1:2. The observation is 13 to 19. The chi-square value of the deviation from expectation equals 0.74 and is not significant.

Double exchanges which have occurred in attached-X females bearing an autosomal inversion have been considered in the same fashion. From the progeny tests of wild type flies, the number of 2-strand, 3-strand, type-1 and type-2 double exchanges are obtained. The results of the progeny tests are enumerated in table 1 and the frequencies of the double exchanges in question have been collected from this table and summarized in table 4. The type-1 and type-2 double crossover types which are homozygous for one or more recessive mutants are listed in table 3 and are also summarized in table 4.

When the first exchange is reciprocal, the observation is 6 of the 2-strand type and 5 of the 3-strand type. When the first exchange is non-reciprocal, the observation is 7 of the type-1 and 8 of the type-2. To this total of 15 can be added the type-1 and type-2 doubles obtained by progeny testing flies which were homozygous for one or more mutant alleles and which had been derived from a double exchange as indicated by their phenotype. There were 23 cases of the type-1 double and 17 cases of the type-2 double. These 40 additional flies plus the previously obtained 15 flies gives a total of 55. Of this total, 30 were derived from the type-1 double exchange and 25 were derived from the type-2 double exchange. The deviation from an expectation of 1:1 is not significant.

It was pointed out earlier when we were discussing the frequency of the two types of single exchanges that the occurrence of doubles introduced a bias into our expected 1:1 ratio. If only the rightmost crossover was noted in the case of a double exchange, the bias was in the direction of an excess of non-reciprocal exchanges. The explanation for that statement can now be given. If doubles occur at random and only the rightmost exchange is noted, then the expectation of reciprocal to non-reciprocal single exchanges is 4:5. Observation of figure 2 will clarify this statement. Since only wild type flies were progeny tested, the types (1), (3), (7), and (8) would be observed and listed as reciprocal exchanges. The types (6), (10), (12), (14), and (16) would correspond to non-reciprocal first exchanges. The ratio is 4 reciprocal to 5 non-reciprocal. Hence when doubles do occur, the expected 1:1 ratio will be biased toward a 4:5 ratio to an extent which is dependent upon the frequency of double exchanges. Table 4 indicates that double exchanges were not very frequent and could not cause an appreciable deviation from a 1:1 ratio in this data.

THE CALCULATION OF CROSSING OVER PERCENTAGES

If one utilizes the material summarized in table 1 and table 5, it is possible to estimate the exchanges which occurred between any two loci. Table 1 lists the results of successful progeny tests on 1638 of 2006 wild type flies; table 5 lists the various frequencies of the 451 flies which had become homozygous for one or more mutant alleles. For purposes of the calculation it has been assumed that all 2006 wild type flies survived the progeny tests, and that the frequency of their various genotypes can be accurately estimated from the 1638 successful progeny tests. The crossover values are therefore based on 2006 + 451 = 2457 flies or 4914 chromosomes since each female carried two tested chromosomes.

TABLE 5

A summary of the attached-X offspring which had become homozygous for one or more mutant alleles

Phenotype of offspring	Parents +/+* number	Parents D/+† number	Phenotype of offspring	Parents +/+* number	Parents D/+† number
ptg v m g sd f	48	26	*m g sd f*	4	5
ptg v m g sd	47	12	*m g sd*	1	6
ptg v m g	55	70	*m g*	2	4
ptg v m	82	65	*m*	0	0
ptg v	57	20	*g sd f*	3	9
ptg	116	85	*g sd*	2	1
v m g sd f	15	14	*g*	1	1
v m g sd	8	4	*sd f*	1	10
v m g	5	13	*sd*	0	1
v m	2	1	*f*	1	4
v	1	0	*ptg sd f*	0	1
			Total	451	352

* *In(3)DcxF* not present.

† *In(3)DcxF* present.

TABLE 6

Crossover frequencies in attached-X chromosomes

	plg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	*f-sp.f.**	Total
Non-inversion attached-X	10.8	4.5	6.7	5.4	5.1	5.5	38.0
Inversion attached-X	12.2	3.4	9.8	8.1	4.4	8.1	46.0
Standard	9.8	3.1	8.3	7.1	5.2	9.3	42.8

* Based on the frequency of non-reciprocal exchanges.

The exchanges which occurred in the exceptional interval forked to the centromere had to be calculated by summing all the observed exchanges and multiplying the value by two. This procedure had to be followed because only the non-reciprocal exchanges yield chromosomes which are known to result from an exchange in this region. The reciprocal exchanges which occur cannot be identified. On the basis of the assumption that reciprocal and non-reciprocal exchanges occur in equal frequency, the total number of exchanges should equal twice the number of observed non-reciprocal exchanges.

The calculated crossover frequencies along with the standard frequencies are listed in table 6. In general it is the regions near the centromere which deviate the most and these deviations are in the minus direction. This is the same phenomenon which was observed by BEADLE and EMERSON (1935). In fact the crossover values calculated here are quite similar to values observed by these two investigators.

FREQUENCIES OF HOMOZYGOSIS

Since a non-reciprocal exchange results in homozygosity of loci to the left of such an exchange, it is apparent that the frequency of homozygosity of any particular locus is a function of the frequency of exchanges which occur between that locus and the centromere. A non-reciprocal exchange yields with equal frequency homozygosity for either the mutant alleles or the wild type alleles; therefore it is possible to obtain two homozygosis values for each locus. The homozygosis values of the wild type alleles can be obtained from table 1; the values for the mutant alleles are obtained from table 5. For example, in table 5 there are listed 72 cases of homozygosity for forked. These 72 cases appeared among the 2457 offspring of the attached-X parents. The frequency of homozygosis therefore equals 2.93%. The homozygosis value of the wild allele of the forked locus is a little more difficult to obtain. In table 1 are listed 52 cases of homozygosity out of a total of 1638 successful progeny tests of wild type flies. There was a total of 2006 wild type flies out of 2457 total progeny. Therefore, the frequency of homozygosity in the 2006 wild type flies would have been $52/1638 \times 2006$. The homozygosis frequency of the total of 2457 flies would therefore equal $52/1638 \times 2006/2457 = 2.59\%$. In a similar manner the homozygosis frequencies have been calculated for each allele. The frequencies are listed in table 7 for the case where the female parents are free of the inversion, and for the case where the parents are heterozygous for the autosomal inversion.

TABLE 7

Homozygosis frequencies of wild and mutant alletes

		plg	*v*	*m*	*g*	*sd*	*f*
Non-inversion attached-X	Wild alleles	16.7	12.5	10.7	7.8	4.7	2.6
	Mutant alleles	16.5	13.2	10.9	7.8	5.2	2.9
Inversion attached-X	Wild alleles	17.7	15.2	14.0	9.2	5.7	3.8
	Mutant alleles	17.6	14.2	13.8	10.4	5.6	4.3

TABLE 8

A summary of exchange and non-exchange chromatids carried by attached-X females

	Non-crossover	%	Single crossover	%	Double crossover	%	Total
+/+*	2352	71.8	896	27.4	28	0.9	3276
D/+†	1171	67.5	539	31.1	24	1.4	1734
χ^2	2.93		5.51		3.06		11.50

* *In(3)DcxF* not present.
† *In(3)DcxF* present.

DISCUSSION

Table 2 contains the pertinent data relative to a discussion of single exchanges. We have already seen that reciprocal and non-reciprocal exchanges occur with equal frequency whether or not the inversion is present; how then do these two experiments differ otherwise? As might be expected, exchanges between chromatids are more frequent in the presence of the inversion. Table 8 furnishes a basis for this statement. The chromosomes carried by each attached-X female listed in table 1 have been considered singly and independently of each other; they have been listed as non-crossover, single crossover, or double crossover chromosomes. Once this operation was completed, a homogeneity test was performed, the results of which are summarized in table 8. The results of the two experiments are not homogeneous. When the inversion is present, the single and double crossover strands increase in frequency and the non-crossover strands decrease in number.

Another question that arises concerns the distribution of crossovers along the marked portion of the chromosome. Do the exchanges in the attached-X have the same distribution whether or not an autosomal inversion is present? The answer to this question can be found by referring to table 9 which contains a summary of a homogeneity test between these two sets of data. The chi-square value of 17.65 for 4 degrees of freedom corresponds to a P value of less than 0.01. The distribution of exchanges is not the same in the two experiments. It is obvious that the greatest portion of the chi-square value is caused by discrepancies between vermilion and miniature, and between miniature and garnet. The contribution by these two regions is equal to 15.54; furthermore, the divergence of these two regions is in opposite directions. There are too many exchanges in one section of the chromosome and too

TABLE 9

A comparison of the regional distribution of exchanges in attached-X chromosomes

	Region of Crossover					Total
	pig-v	*v-m*	*m-g*	*g-sd*	*sd-f*	
+/+*	315	129	194	165	149	952
D/+†	190	51	162	108	76	587
χ^2	0.06	7.37	8.17	0.24	1.81	17.65

* *In(3)DcxF* not present.

† *In(3)DcxF* present.

few in the neighboring section. If we combine the two regions into one region within the markers vermilion to garnet (enclosing a standard map unit distance of 11.1) and perform another homogeneity test with 3 degrees of freedom, the significance disappears.

From these observations it is possible to conclude that if the only genetic markers in this region had been vermilion and garnet, one would have been reasonably convinced that the inversion had no effect upon the distribution of exchanges in this section of the chromosome. One wonders if other regions of the chromosome are not reacting in a similar fashion but that this has not been detected because the marked portions of the chromosome have been too gross.

STEINBERG (1936), STEINBERG and FRASER (1944), SCHULTZ and REDFIELD (1951), and MORGAN, REDFIELD and MORGAN (1943) have all reported regional differences in crossing over under the influence of an inversion. In a few cases at least the frequency of exchanges was about standard in one region and considerably greater in a close neighboring region. With observations such as these already on record, it does not seem too presumptuous to suggest that an inversion may cause regional inhibitions as well as regional increases in crossover frequencies (table 6).

It is interesting to note a possible correlation between these observations and the distribution of euchromatic and heterochromatic sections of the salivary chromosome. HANNAH (1951) has summarized a great deal of work on the localization of heterochromatin. From her summary it appears that none of the investigators who have used the phenomena of high breakability or ectopic pairing as criteria for the localization of intercalary heterochromatin have claimed a localization within the region 9C to 10F of the salivary gland chromosome. BRIDGES (1938) places the locus of vermilion at 10A of the salivary map and DEMEREC, KAUFMAN, FANO, SUTTON, SANSOME (1942) place miniature somewhere between 10C3–4 and 10E1–2. Both of these loci appear to lie within a portion of the salivary chromosome which seems to be free of intercalary heterochromatin. A discussion of Inversion(1)delta-49 found in BRIDGES and BREHME (1944) suggests that the locus for garnet lies beyond the region 11F4, possibly in region 12 or 13 of the salivary map. From the summary by HANNAH (1951) it appears that region 11 and 12 of the salivary map both contain heterochromatin. Therefore, it is possible that the region vermilion to miniature lies within a portion of the chromosome which is primarily euchromatic, and the region miniature

to garnet differs from the previous region by containing abundant intercalary heterochromatin.

One might now postulate that the increased frequency of crossing over between miniature and garnet is due to a stimulatory effect of the Dichaete inversion upon the intercalary heterochromatin localized between these two loci. The decrease in crossing over between vermilion and miniature may be due to an inhibitory effect of the inversion upon the euchromatin localized in this region of the chromosome. The observation upon which this postulate rests will have to be carefully checked.

It has been indicated earlier that double exchanges occur as expected whether or not an autosomal inversion is present; therefore, it is possible to combine the data. If we compare the double exchanges in which the rightmost exchange is reciprocal to those cases in which the first exchange is non-reciprocal, the observation becomes 24 to 34. The expected ratio is 1:2. A chi-square test yields a value of approximately 1.7 which is not statistically significant. The two types of doubles in which the first exchange is non-reciprocal are expected to occur in a ratio of 1:1. The observed numbers are 93 to 84 and the deviation from expectation is obviously not significant. The totals given by BEADLE and EMERSON (1935) may be added to this to give a grand total of 245 to 205. This total approaches significance at the five percent level.

A more detailed analysis of the data shows an interesting trend which has not been emphasized yet. If one enumerates all those double exchanges which have taken place within a short segment of the marked region of the chromosome, as opposed to those exchanges which have occurred within a larger region of the chromosome, it appears that there is a preponderance of the type-1 exchanges over the type-2 exchanges. In table 4 all those individuals with a vermilion, miniature, garnet, or scalloped phenotype have resulted from a double exchange within a shorter region of the chromosome than any of the other phenotypes listed in this table. Where the double exchanges have taken place in these short regions, the observation is 22 of type-1 to 7 of type-2. A 1:1 ratio is expected. A few more of these types of double exchanges are listed in table 1. They bring the total observation to 22 type-1 to 10 type-2. A chi-square test (with the application of Yates' correction) yields a value of 3.78. The difference between observation and a 1:1 expectation is on the borderline of significance at the five percent level. More data are necessary before a good demonstration of negative chromatid interference can be claimed.

It is possible to interpret this observation so that it is consistent with the theory of sister strand crossing over as stated by SCHWARTZ (1953). If meiotic exchanges occur only between the newly formed chromatids, as first postulated by BELLING (1931), then it follows that in the case of a double exchange both exchanges would involve the same two chromatids and would result in a 2-strand double crossover in which the first exchange was reciprocal. If a sister strand exchange occurred in one of the arms to the right of the first meiotic exchange, or an odd number of sister strand exchanges occurred as the sum of all such exchanges in both arms, the 2-strand double in which the first exchange was reciprocal would be converted into a 2-strand double in which the first exchange was non-reciprocal. Under those conditions where the meiotic crossovers are far apart, sister strand crossing over may occur between the two initial meiotic exchanges and destroy the appearance of the 2-strand double type. However,

as the two meiotic exchanges occur closer and closer together, it could be that there is less and less opportunity for a sister strand exchange to occur between them. It follows that under such conditions one would eventually obtain an excess of the 2-strand double type (type-1).

The work discussed here agrees in general with the work performed by BEADLE and EMERSON and disagrees sharply with that of BONNIER and NORDENSKIÖLD (1937). The latter investigators claimed to have demonstrated a significant excess of non-reciprocal single exchanges and, in addition, the action of chromatid interference. The data presented in this paper and the work of BEADLE and EMERSON indicate that single exchanges occur at random and if there is any chromatid interference, it must be of the negative type. The discussion below indicates that the data of BONNIER and NORDENSKIÖLD cannot be seriously considered as contradictory to the conclusions formulated here.

In accumulating the single exchanges, BONNIER and NORDENSKIÖLD multiplied all non-reciprocal exchanges by two because such an exchange yields homozygosity for both wild alleles and mutant alleles, and for every homozygous wild type fly recovered, there must have been a corresponding homozygous mutant type. On this basis non-reciprocal exchanges would be twice as frequent as reciprocal exchanges so reciprocal exchanges were then multiplied by two and a chi-square test was applied on the basis of a one to one expectation of reciprocal to non-reciprocal. In this way the chi-square values were doubled, and since they appeared to be significant, the investigators concluded that there was an excess of non-reciprocal exchanges. If the chi-squares are divided by two, the significance disappears.

If one uses the raw data presented by these investigators and enumerates the reciprocal and non-reciprocal exchanges following the method used by this author, there is an excess of non-reciprocal exchanges on the basis of a 1:1 expectation. However, it was pointed out earlier that if double exchanges become frequent, the 1:1 ratio becomes biased in the direction of a 4:5 ratio of reciprocal to non-reciprocal types. Since these investigators were studying practically the whole of the X-chromosome, from yellow to forked, double exchanges were frequent and consequently there should be found an excess of the non-reciprocal type. The actual observation lies between a 1:1 and 4:5 ratio as expected.

BONNIER and NORDENSKIÖLD concluded that there was an excess of 4-strand double exchanges and their raw data do indicate a large excess of the type-2 double. However, in many instances the double exchange which occurred had removed so many of the intervening markers that the progeny test could not determine accurately whether the remaining markers were on the same or different chromosomes. For example, a double crossover in which the first exchange is non-reciprocal and is located just to the left of forked and the remaining exchange is just to the right of yellow will yield an attached-X complex which is heterozygous for only yellow and forked, but the markers will be so far apart that the progeny test will be unable to determine whether or not they are on the same chromosome. In fact, type-1 doubles in which yellow and forked are on the same chromosome will be misclassified as type-2 doubles in which yellow and forked are on different chromosomes. It is very probable that at least some of the excess 4-strand doubles are due to a misclassification of this type.

There is one remaining point to be made. If we compare doubles in which the first exchange is reciprocal with those in which the first exchange is non-reciprocal, a 1:2 ratio is expected if doubles occur at random. From the data of BONNIER and NORDENSKIÖLD one can extract 27 of the first type and 73 of the second. A chi-square on the basis of a 1:2 expectation is not significant. If chromatid interference were at play, we would have expected a suppression of the class in which the first exchange is reciprocal because all of this class results from 2-strand and 3-strand doubles. A bias toward 4-strand doubles would rob this class and leave it deficient. If there is any chromatid interference put in evidence by these data, it must come into play only when the first exchange of a double is of the non-reciprocal type.

SUMMARY

In the course of this experiment, data have been collected which yield information on the process of crossing over as it occurs in attached-X females of *Drosophila melanogaster*. Two types of parental females were used. In one case the parents were heterozygous for the third chromosome inversion *DcxF*, and in the other case the inversion was not present. The attached-X chromosomes were heterozygous for pentagon^3, vermilion, miniature, garnet^2, scalloped, and forked; all the recessive mutants were located in one chromosome. The pertinent observations were as follows:

1. In the case of single exchanges one would expect the reciprocal and non-reciprocal type of crossover to occur with equal frequency if crossing over is at random between non-sister chromatids. A 1:1 relationship was demonstrated between the two types of exchanges. When the autosomal inversion was present in the parental females, the ratio of reciprocal to non-reciprocal exchanges remained the same, but the total exchange frequency was increased.

2. In the presence of the inversion, the distribution of exchanges along the marked segment of the chromosome was altered. In the region from vermilion to miniature (3.1 map units) the exchange frequency was markedly reduced while in the neighboring region miniature to garnet (8.3 map units) the frequency was greatly increased. It was indicated that the first region is primarily a euchromatic region while the region miniature to garnet appears to contain appreciable intercalary heterochromatin. This suggests that the inversion has stimulated the heterochromatin to crossover while crossing over in the euchromatin has been inhibited.

3. In general, the expected ratios of various types of double exchanges were obtained. These same ratios were observed when the parental females were heterozygous for the inversion. It was noticed, however, that as two exchanges occur at points which are closer and closer together, there is a tendency to obtain an excess of 2-strand double exchanges over the 4-strand type; *i.e.* there appears to be a negative chromatid interference under these conditions.

4. The observation of an excess of 2-strand double exchanges was shown to be consistent with the theory of sister strand crossing over.

5. The data presented in this paper are in reasonable agreement with the data of BEADLE and EMERSON but disagree sharply with the data of BONNIER and NORDENSKIÖLD.

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SEGREGATION OF THE SELECTIVE ADVANTAGE OBTAINED THROUGH ORTHOSELECTION IN ESCHERICHIA COLI[1, 2]

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THE existence of an unanticipated selective process in populations of bacteria was postulated by STOCKER in 1948. While engaged in measuring the mutation rate of antigenic variants of Salmonella, he observed that in mixtures predominantly of one phase a fluctuation occurred in the frequency of the other phase. He pointed out that the most probable explanation of this phenomenon is the appearance of a more rapidly growing mutant which displaces its predecessors in the culture.

SCHNEIDER (1950) working independently with mixtures of *Escherichia coli* mutants observed that the frequency of the minority type was constant for a time and then underwent a rapid decline. She demonstrated that a population shift had occurred because of the overgrowth of a mutant with a selective advantage. Similar results were obtained by NOVICK and SZILARD (1950) who observed the repetitive nature of the selection process. A year later ATWOOD, SCHNEIDER and RYAN (1951a) published the results of experiments which demonstrated conclusively the existence of a unique selective mechanism in strain 15 of *E. coli*. They found that in growing cultures consisting predominantly of h^- (histidine dependent) cells there was a cyclical rise and fall in the frequency of the h^+ mutants brought about by the emergence of h^- cells which enjoyed a selective advantage and therefore soon constituted the preponderant type in the culture. h^+ mutants of this new type at once began a gradual increase toward the mutational equilibrium frequency but were interrupted before this was reached by the appearance of a second fitter type among the h^- and the ensuing rapid turnover in the population. To describe this phenomenon they proposed the term "periodic selection" and suggested that this mechanism operated continuously in populations of microorganisms subject to extended periods of growth, but was detectable only when fluctuations in a suitable indicator strain could be observed. Since it is now understood that these fluctuations are only a reflection of a continuous selective process the more accurate term "orthoselection" is currently in use (RYAN 1953).

The changes brought about in the population by orthoselection seemed to be stable and heritable but a direct genetic analysis of the selective advantage was not possible because of the unfortunate lack of a known sexual phase in the strain employed. A survey of the literature immediately suggests several different genetic schemes which might be operating here. Cytoplasmic inheritance has been demonstrated in

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microorganisms (SONNEBORN 1950; EPHRUSSI 1951; MITCHELL and MITCHELL 1952) and it is at least conceivable in the present instance that the conditions imposed by continuous growth over long periods of time might be conducive to the accumulation of extranuclear components which confer a selective advantage upon the cells harboring them. On the other hand the discovery of recombination in *E. coli* K12 (LEDERBERG and TATUM 1946) has led to the conclusion that a nuclear mechanism exists in this bacterium controlling the inheritance of many different traits. Instances of single gene (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951) and polygenic inheritance (CAVALLI 1952) are known and it is suggestive in the latter instance that long periods of growth under highly selective conditions are required to produce the altered genotype. In any event, since the mechanics of inheritance in *E. coli* were sufficiently clear and a distinction between the various schemes could be made, it seemed feasible to undertake a genetic analysis of the fitter types generated through orthoselection.

EXPERIMENTAL PROCEDURES

It seemed desirable to use material which was as genetically homogeneous as possible, and for this reason a single strain, W1896, kindly supplied by DR. JOSHUA LEDERBERG, was used to produce two crossable substrains by spontaneous mutation of its markers. By the same method five additional unselected marker differences were obtained to provide a basis for a genetic analysis of a polygenic system if this proved to be the case. The genealogies of the strains are summarized in figure 1. Mutants were obtained by plating on selective media and picking a colony. All the fermentation mutants were characterized on a complete indicator agar (EMB without lactose) to which the appropriate sugar had been added. The resulting substrains R4123 and R1131 were relatively infertile under the usual crossing conditions (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951) but produced prototrophs abundantly when aerated cultures, grown in YECA for 12 to 18 hours at 37°, were mixed together 4 to 6 hours, washed twice in saline, resuspended and plated in minimal agar supplemented with 0.01 μg thiamine hydrochloride per ml and 100 μg streptomycin per ml. In control experiments of R4123 plated by itself only a few streptomycin resistant (S^r) colonies per 10^{10} bacteria were found, while R1131 under similar conditions yielded no colonies at all.

The production of fitter types involved the serial transfer system employed by ATWOOD, SCHNEIDER and RYAN (1951a). The cells were inoculated into 125 ml erlenmeyer flasks containing 50 ml of Gray and Tatum minimal medium supplemented with 200 μg DL-threonine, 60 μg DL-leucine and 0.01 μg thiamine hydrochloride per ml, and were incubated without aeration at 37°C for 12 hours, when full growth had been attained. A 0.5 ml sample was then removed to a flask of fresh medium, this was incubated for 12 hours, and a 0.5 ml sample was used to inoculate the third serial transfer (ST). This sequence was repeated until the strain had passed through sixty ST. A single colony isolated from each line at this time was designated 60A1 (from the R4123 line) and 60B1 (from the R1131 line).

To determine the selective advantage of the original types R4123 and R1131, the fitter types 60A1 and 60B1, and recombinants from any cross, two standard

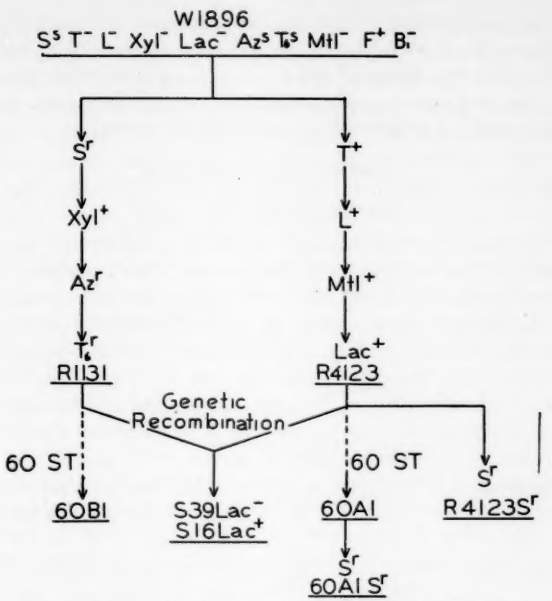

FIGURE 1.—The genealogy of the strains derived from W1896. Thiamine hydrochloride was added to all minimal media so that the B_1 marker need not be considered. Abbreviations for the markers are:

S = Streptomycin	*Xyl* = Xylose	*Az* = Sodium azide
T = Threonine	*Lac* = Lactose	T_6 = Coliphage T_6
L = Leucine	*F* = Mating type	*Mtl* = Mannitol
		B_1 = Thiamine

tester strains were obtained, S39 *Lac*⁻ and S16 *Lac*⁺, as prototrophs from a cross of R4123 and R1131 (fig. 1). *Lac*⁺ strains were tested against S39 and *Lac*⁻ against S16. Testing experiments were begun by inoculating 0.4 ml of the appropriate tester into a ST flask with 0.1 ml of the strain to be tested. This constituted the beginning of a series of eight ST carried out as described in the preceding section. A 0.5 ml sample was removed from the first ST immediately after inoculation, diluted and spread on four EMB plates. Similar samples were removed from each ST at the end of its incubation period. Thus, a set of nine samples was obtained from which any change in the ratio of the two competing strains could be calculated. Assuming that the selective advantage is independent of the ratio of the two types, the log of this ratio plotted as a function of the number of ST is a straight line, and the slope of this selection curve constitutes a comparative measure of the selective value of any strain. In these calculations the ratios were multiplied by ten for convenience in using the log tables.

The growth rate and final level of growth were obtained by the following procedure. Two ml of a stationary culture grown without aeration in ST medium were inoculated

into 400 ml of fresh medium and 50 ml were measured into each of eight 125 ml erlenmeyer flasks. These were allowed to stand undisturbed at 37°C and at appropriate intervals a flask was removed and a 0.5 ml sample was diluted and spread on four plates. The flask was then discarded. In this way the growth occurred under conditions very closely approximating those in the ST system.

RESULTS AND CONCLUSIONS

The cross of the original types

It seemed necessary to establish at the outset that the segregating markers were selectively neutral. For this reason and also to obtain the standards for further tests, the two original strains R4123 and R1131 were crossed and from among one hundred prototrophs picked and characterized, six with different arrays of markers were chosen for further analysis (table 1). A 1:1 mixture of S39 and S16 carried through eight ST underwent no change in proportion, for the slope of the line plotted in figure 2 is not essentially different from zero. Since these two strains were equal in their selective advantage and carried opposite sets of markers (with the exception of mannitol) they were chosen as standards against which all the other strains were subsequently tested. A 1:4 mixture of S125 *vs.* S16 when tested as described above underwent no significant change in the initial ratio (fig. 2). Essentially the same results were obtained with S14 *vs.* S39, S63 *vs.* S39, S25 *vs.* S39 and R1131 *vs.* S16, although here there seemed to be a small but definite rise in the proportion of R1131

TABLE 1

Recombinants from the crosses of R4123 × R1131

Strain*	*S(TL)*	*Xyl*	*Lac*	*Az*	*T*	*Mtl*	Slope
R4123	s +	−	+	s	s	+	0.288
R1131	r −	+	−	r	r	−	0.060
S39	r +	−	−	r	r	−	0.000
S16	r +	+	+	s	s	−	0.000
S63	r +	−	+	s	s	−	0.029
S25	r +	−	+	s	r	−	0.041
S125	r +	−	−	s	s	−	0.022
S14	r +	+	+	s	r	−	0.019
R4123S^r	r +	−	+	s	s	+	−0.400
2-1	r +	−	+	s	s	−	0.000
2-20	r +	+	−	r	r	−	−0.011
2-126	r +	−	+	s	r	−	0.020
2-5	r +	+	+	s	s	−	0.032
2-31	r +	+	+	r	s	−	0.019
2-206	r +	+	+	s	r	−	0.000
2-12	r +	−	+	r	r	−	0.030
2-4	r +	−	−	s	r	−	0.012
2-2	r +	+	−	s	r	−	−0.005
2-9	r +	−	−	r	r	−	0.015

* Recombinants from the first cross are labeled "S" and those from the second cross are labeled "2." Strain R4123S^r is a streptomycin resistant reversion of R4123.

(fig. 3). On the other hand, R4123 in competition with S39 was decidedly superior and rapidly replaced S39 in the ST. This was not entirely unexpected since S^s strains have been shown to have a more rapid growth rate than S^r (CAVALLI 1952). The effect of the streptomycin locus in this case was clearly demonstrated, for a S^r reversion of R4123 (R4123S^r) when pitted against S39 now proved to be inferior (table 1). These results were confirmed by a second experiment in which ten recombinants were analyzed (table 1). All were found to be without any selective advantage over the tester. It was concluded from this that the markers were selectively neutral

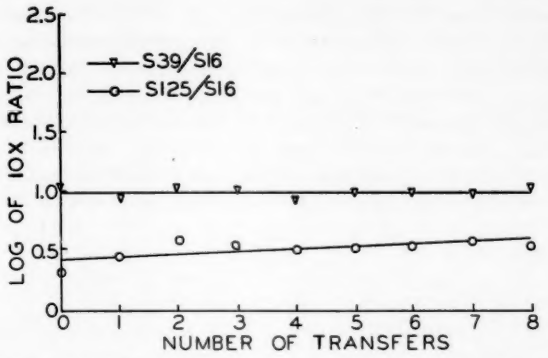

FIGURE 2.—The log of 10× the ratio plotted as a function of the number of serial transfers.

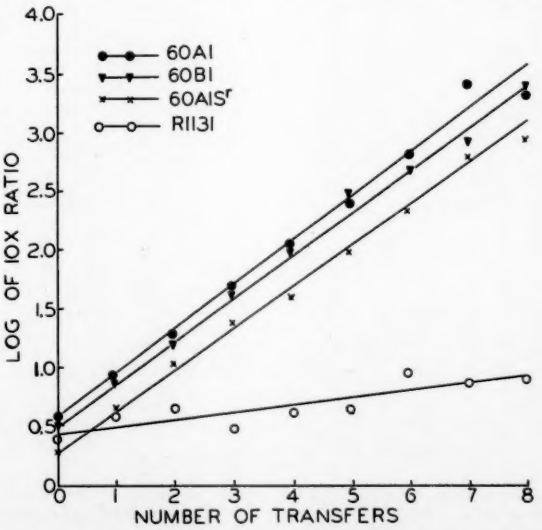

FIGURE 3.—Selection curves for 60A1, 60B1, 60A1S^r and R1131.

with the exception of the *S* locus, and since S^r recombinants apparently did not inherit the rapid growth rate of the S^s parent, the effect of this locus could be disregarded.

The selective advantage of the fitter types

When 60A1 and 60B1 were placed in competition with the standards it became at once evident that during the course of the serial transfers the original strains had acquired a pronounced selective advantage (fig. 3). A comparison of the slope of the R1131 selection curve with that of 60B1, the fitter type derived from it, reveals a six-fold difference which is definitely significant. A S^r reversion of 60A1 (60A1S^r) proved to have the same slope as 60A1 itself, thus demonstrating that the S^s locus was not contributing to the selective advantage of this fitter type. It is interesting that the slopes of 60A1 and 60B1 are nearly identical and, in fact, no significant difference between them can be demonstrated This fact suggests that changes which occurred during the evolution of the fitter types may have been the same in the two strains despite the fact that they were evolved independently of each other. The test of this hypothesis involves the crossing of the fitter types with the original strains

TABLE 2

Recombinants from the crosses of 60A1 × R1131

Strain*	*S*(*TL*)	*Xyl*	*Lac*	*Az*	*Ts*	*Mtl*	Slope
60A1	s +	−	+	s	s	+	0.381
R1131	r −	−	−	r	r	−	0.060
0-37	r +	+	−	s	s	−	0.245
0-292	r +	−	−	s	s	−	0.225
0-45	r +	+	+	s	s	−	0.210
0-108	r +	−	+	r	s	−	0.299
0-99	r +	−	−	s	r	−	0.297
0-20	r +	+	+	r	s	−	0.015
0-233	r +	+	−	s	s	−	0.000
0-5	r +	+	−	s	r	−	0.017
0-13	r +	−	−	r	r	−	0.018
0-18	r +	+	+	r	r	−	0.012
60A1S^r	r +	−	+	s	s	+	0.321
1-27	r +	−	+	s	s	−	0.026
1-84	r +	+	+	s	s	+	0.238
1-284	r +	+	+	r	s	+	0.166
1-5	r +	+	−	r	r	−	0.019
1-75	r +	+	+	s	s	−	0.035
1-133	r +	−	−	s	r	+	−0.023
1-293	r +	+	+	r	s	−	0.020
1-24	r +	−	−	s	r	−	0.024
1-120	r +	+	+	s	r	−	0.200
1-13	r +	+	−	s	r	−	0.029
1-50	r +	−	−	r	r	−	0.022

* Recombinants from the first cross are labeled "0" and those from the second cross are labeled "1." Strain 60A1S^r is a streptomycin resistant reversion of 60A1.

and with themselves, and observing the segregation of the selective advantage among the recombinants.

The selective advantage of the recombinants

Ten recombinants with different markers were isolated from a cross of 60A1 with R1131 and the selective advantage of each was determined (table 2). In figure 4, the slopes of the selection curves for each, calculated by regression, are used to construct lines radiating from a common point. It is immediately apparent that the recombinants fall into two distinct classes, five without any selective advantage over the tester, and five with an intermediate advantage. There are none with a selective advantage as high as 60A1. A significant difference ($t = 11.16$, $P < 0.005$) can be shown to exist between the lowest curve in group A and the highest curve in group B. Within group B no differences among the curves can be demonstrated, while in group A probably only the curves at the extremes of the group are different. ($t = 2.93$, $P = 0.005$).

When this experiment was repeated and eleven new recombinants were tested (table 2 and fig. 4) the same general pattern prevailed. In this case there were three with an intermediate selective advantage and eight with no selective advantage. Since there is no difference between the two sets of data ($\chi^2 = 0.014$, $P = 0.90$) the results can be pooled.

On the basis of these experiments a tentative hypothesis of the genetics of the selective advantage in 60A1 could be constructed. The segregation of the selective

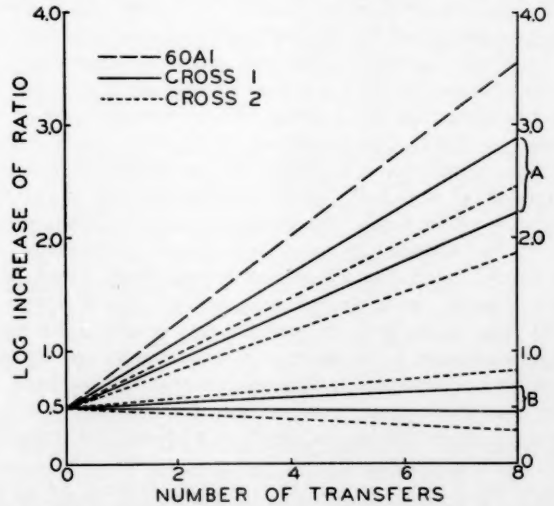

FIGURE 4.—Selection curves plotted from a common point of recombinants from the crosses of 60A1 × R1131. Only the curves at the upper and lower limits of each group are drawn. Data are given in table 2.

TABLE 3

The segregation of the selective advantage with the markers in the crosses of the filler types

Selective advantage	*Xyl*		*Lac*		*Az*		*T*$_6$		*Mtl*	
	+	−	+	−	s	r	s	r	+	−
60A1 × R1131										
Intermediate	4	4	6	2	6	2	6	2	2	6
None	8	5	5	8	7	6	5	8	1	12
60B1 × R4123										
Intermediate	3	0	2	1	3	0	2	1	2	1
High	8	9	6	11	11	6	9	8	1	16
The segregation of the markers among 221 recombinants										
Percent	56	44	30	70	80	20	28	72	0.5	99.5

advantage into distinct classes indicates the presence of discrete heritable entities whose distribution is discontinuous and controlled. It is difficult to reconcile the segregation pattern obtained with that expected where the trait is cytoplasmic, polygenic or caused by a single gene. The simplest hypothesis which embodies all the facts assumes the existence of two genes which determine the degree of selective advantage. If these two genes are not linked to each other, the unselected markers or the selective markers S and TL, then three classes of segregants would be expected: a group in which each segregant carries both genes and consequently possesses as high a selective advantage as 60A1, a group with only one of the genes and an intermediate selective advantage, and a group with neither gene and no selective advantage, similar to R1131. The proportion of the three classes would be 1:2:1, respectively. Upon comparison it is obvious that the proportion obtained is different from that expected ($\chi^2 = 17.3$, $P < 0.001$) and therefore that the genes in question are not segregating independently. The segregation pattern of the unselected markers was similar to that obtained by other investigators (LEDERBERG, LEDERBERG, ZINDER and LIVELY 1951). The *Mtl* marker seemed closely linked to the *S* locus, with *Lac* and *T*$_6$ nearby, while *Az* was closer to (*TL*). Xylose seemed independent of both *S* and (*TL*). It can be shown from the data in table 3 that the segregation of the selective advantage is not appreciably influenced by the segregation of the unselected markers. Consequently, although the recombinants were chosen with regard to their markers the sampling method was essentially random so far as selective advantage was concerned and has not significantly affected the segregation pattern. The analysis of this pattern suggests that both genes are linked to each other and are in the region of the chromosome near the streptomycin locus, for in the *S*^{s} parent 60A1, this is the region against which selection operates during the crossing procedure. The formation of a segregant without any selective advantage requires no crossing over between the postulated genes and the *S* locus. These segregants therefore are more frequent than those with intermediate selective advantage, which require at

TABLE 4

Recombinants from the crosses of 60B1 × R4123

Strain*	*S(TL)*	*Xyl*	*Lac*	*Az*	*Ts*	*Mtl*	Slope
60B1	r −	+	−	r	r	−	0.370
3-64	r +	−	+	s	s	−	0.352
3-26	r +	−	−	s	s	−	0.382
3-75	r +	+	+	s	s	−	0.243
3-1	r +	−	−	s	r	−	0.327
3-143	r +	+	−	s	s	−	0.395
3-263	r +	+	+	r	s	−	0.375
3-6	r +	−	−	r	r	−	0.357
3-4	r +	+	−	s	r	−	0.328
4-11	r +	+	+	s	s	+	0.209
4-13	r +	−	+	s	s	−	0.375
4-359	r +	−	−	s	s	−	0.401
4-53	r +	−	+	r	s	−	0.395
4-52	r +	+	+	s	s	−	0.343
4-35	r +	+	+	r	s	−	0.371
4-241	r +	+	−	s	r	+	0.221
4-2	r +	−	−	s	r	−	0.334
4-3	r +	+	−	s	r	−	0.354
4-17	r +	−	−	r	r	−	0.386
4-14	r +	+	−	r	r	−	0.351

* Recombinants from the first cross are labeled "3" and those from the second cross are labeled "4".

least one crossover, or recombinants with a high selective advantage which possibly involve two chromosome breaks. Indeed, on this assumption the last class should be, and is, the most infrequent of the three, for none of this type was recovered in either cross.

Before any further work was done on 60A1, the second fitter type 60B1 was crossed with R4123 and eight recombinants were analyzed (table 4). Upon comparing their slopes (fig. 5) it was seen that only two classes of segregants had appeared, one group (C) consisting of seven isolates with high selective advantage about equal to that of 60B1, and the second group (A) containing only one isolate with an intermediate selective advantage. Recombinants with no selective advantage were not obtained (fig 5). This cross was repeated and yielded nine recombinants with high selective advantage, two intermediates and again none without any selective advantage (table 4 and fig. 5). The results of the two experiments were not significantly different ($\chi^2 = 0.0001$, $P = 0.99$) and could be combined. It is interesting to observe that these results confirm the general conclusions reached concerning the inheritance of selective advantage in 60A1. Furthermore, the simplest hypothesis which can be formulated to explain the proportion of the segregants obtained is the same as that proposed for 60A1, that is, two genes linked to the streptomycin locus. If the genes are allelic the cross 60B1 with R4123 should be the reciprocal of the cross 60A1 with R1131. This assumption is vindicated when the data from the two crosses are compared (table 5) for no significant difference can be demonstrated ($\chi^2 = 2.51$, $P = 0.21$).

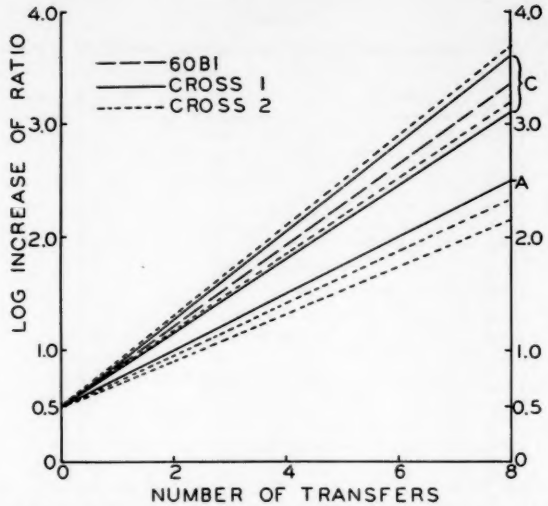

FIGURE 5.—Selection curves plotted from a common point of recombinants from the crosses of 60B1 × R4123. Only the curves at the upper and lower limits of the higher group are drawn. Data are given in table 4.

TABLE 5

A comparison of the segregation of the selective advantage in the two fitter types

Cross	Degree of selective advantage		
	None	Intermediate	High
60A1 × R1131	13	8	0
	High	Intermediate	None
60B1 × R4123	16	3	0

Data in first two columns used for test of independence between segregation ratio of the crosses 60A1 × R1131 and 60B1 × R4123. $\chi^2 = 1.49$, $P = 0.22$.

An additional test of the hypothesis of allelism was possible, for if the two fitter types are crossed with each other all the segregants should have a high selective advantage. Ten segregants from such a cross were analyzed and all possessed a selective advantage about the same as that of 60A1 and 60B1 (table 6). This experiment was repeated and the analysis of ten additional segregants gave similar results (table 6).

The nature of the selective advantage

A study of the nature of the selective advantage in 60B1 was made by comparing the individual growth characteristics of this fitter type with those of the original type R1131. In figure 6 the combined results of two separate determinations of the

TABLE 6

Recombinants from the cross of 60A1 × 60B1

Strain	*S(TL)*	*Xyl*	*Lac*	*Az*	*T6*	*Mtl*	Slope
60A1	s +	−	+	s	s	+	0.381
60B1	r −	+	−	r	r	−	0.370
5-2	r +	+	−	s	r	−	0.306
5-25	r +	+	−	r	s	−	0.346
5-1	r +	+	+	s	s	−	0.331
5-53	r +	+	+	s	r	−	0.311
5-10	r +	−	+	s	s	−	0.327
5-6	r +	−	+	r	s	−	0.303
5-22	r +	−	−	r	r	−	0.333
5-5	r +	−	−	s	r	−	0.347
5-99	r +	−	+	s	r	−	0.361
5-126	r +	+	−	s	r	+	0.369
6-1	r +	−	+				0.300
6-2	r +	+	−				0.341
6-3	r +	+	+		Markers		0.361
6-4	r +	−	−				0.363
6-5	r +	−	+		not		0.383
6-6	r +	−	+				0.303
6-7	r +	+	−		analyzed		0.320
6-8	r +	−	+				0.400
6-9	r +	−	−				0.350
6-10	r +	+	−				0.333

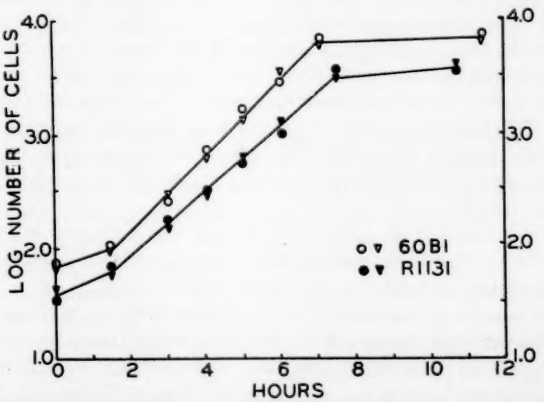

FIGURE 6.—Growth curves of R1131 and 60B1. The combined results of two separate experiments are plotted for each strain.

growth curve are plotted for 60B1 and R1131. In both experiments the generation time of 60B1 was lower (54 and 55 minutes) than that of R1131 (64 minutes) and the final level of growth was higher (5.9×10^8 and 5.6×10^8 per ml) for 60B1 than for R1131 (3.0×10^8 and 4.0×10^8 per ml). The differences in generation time and

plateau are significant and it seems very probable that one or both factors are responsible for the selective advantage of 60B1. The assumption that the critical factor is the difference in generation time can be tested by constructing a theoretical selection curve. This is done by plotting the growth curves of both strains simultaneously, with the initial number of R1131 four times that of 60B1. This of course is the theoretical situation under which the selection curves for all the strains were obtained. When the total number of cells has increased one hundred times this point represents the end of the first ST, and each succeeding one hundred-fold increase marks the end of the following transfer. These points can be estimated from the graph and at each the ratio of 60B1/R1131 is obtained. The log of this ratio plotted as a function of the numbers of transfers produces a straight line with a slope 0.330 which compares favorably with the experimental slopes of 60B1 (0.375 and 0.345). On the other hand, if the plateau difference is used to construct a theoretical selection curve the value obtained for the slope is rather high (0.45 using the lower generation time), and when both plateau and generation time differences are superimposed the value becomes higher still (0.55). It seems probable therefore that in the mixtures the plateau difference does not have the opportunity to manifest itself.

DISCUSSION

A number of interesting comparisons can be made between the results obtained by ATWOOD, SCHNEIDER and RYAN (1951b) and those presented in this paper. They demonstrated that when the selective advantage is used as the comparative measurement, fitter types obtained independently are frequently different from each other, and it is reasonable to suppose that these differences reflect underlying genetic dissimilarity. In this instance the genetic basis of the selective advantage obtained independently in the two strains is apparently the same. It will be necessary to analyze the genetics of many more fitter types before any general conclusions can be drawn, but it does not seem unlikely that parallel fitter types will frequently carry allelic genes. This may be an effect of differences in mutation rates among the genes concerned with selective advantage, for if such differences exist, those mutants which arise more frequently will have a much better chance of establishing themselves during a serial transfer.

Regarding the nature of the selective advantage, the findings of this investigation are in accord with those of ATWOOD and his coworkers (1951b), who demonstrated growth rate increases in several of their fitter types.

A rough estimation can be made of the time needed for the increase of the fitter type mutants after their appearance in the ST, by using the slope of the selection curve for a type with intermediate selective advantage, 0–37 for instance. If such a curve is plotted it can be estimated that a ten-fold increase in the ratio occurs during every four ST. This means that about 32 transfers may have been necessary to increase the mutant ratio from 10^{-8} to 1. Unfortunately it is not possible to estimate by this method the number of transfers necessary in the second cycle for there is no data available yet on the selection curves of mixtures of high and intermediate fitter types. However, it is possible to estimate the growth rate of an intermediate type, and from this a theoretical selection curve of intermediate *vs.* high type can be con-

structed. The slope of this curve indicates a minimum of 44 ST to bring about an increase of 10^{-8} to 1. These values for the number of transfers seem to be rather high when compared with the findings of ATWOOD, SCHNEIDER and RYAN (1951a) who demonstrated that about 30 ST were necessary for their fitter types to dominate the culture. Furthermore, it is particularly interesting to note in the present instance that while 76 transfers is the minimum estimate of the number required to establish the final fitter type, 60B1, only 60 ST were actually carried out. This difference of course may be due to inaccuracies introduced by the approximations, or to a higher mutation frequency than that assumed. A more tempting possibility is that the final fitter type was brought into existence by genetic recombination occurring during the serial transfers. Thus, it is not necessary to assume that cells with both selective advantage genes would begin to appear only after cells with one of the genes had increased through 32 ST. Undoubtedly both single mutations occur quite early in the course of the ST, and if the means exist of bringing them together in a single cell the final fitter type will appear much sooner than expected. In essence, what this means is that recombination will increase the genetic variability upon which the selection process is operating, a fact which is axiomatic in population genetics.

Preliminary experiments indicate that recombination does occur during the course of the serial transfer, and the effect of this factor on the expression of orthoselection is under consideration.

SUMMARY

The results of these experiments indicate that in two strains of *E. coli* allowed to evolve independently, orthoselection has established a pair of genes which confer a high selective value on the cells in the populations. Furthermore, it seems that the genes in the two strains are allelic and linked to the streptomycin locus. The adaptive changes can probably be attributed to an increased growth rate.

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THE BEHAVIOR OF AN UNSTABLE RING CHROMOSOME OF DROSOPHILA MELANOGASTER[1, 2]

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RING-SHAPED chromosomes provide an opportune situation for the analysis of some aspects of chromosome mechanics. In particular, sister-strand crossing over eludes detection in rod chromosomes but its consequences in ring chromosomes are manifested by the formation of anaphase bridges. Thus McCLINTOCK (1938) and SCHWARTZ (1953) have presented cytological evidence for the occurrence of exchanges between sister chromatids of ring chromosomes in mitotic and meiotic divisions in maize. On the other hand, genetic studies on the ring-X chromosomes of *Drosophila melanogaster* apparently deny the occurrence of meiotic sister-strand exchange (MORGAN 1933; NOVITSKI 1955), and although ring-X chromosomes do experience somatic loss more frequently than their rod-X homologs (MORGAN 1926; BROWN and HANNAH 1952), they are described as being stable in comparison with maize rings. This report is concerned with an atypical ring-X chromosome characterized by frequent loss and dominant lethality. Results will be presented which suggest that the instability of this chromosome is mediated through anaphase bridge formation and that this process is controlled by some heterochromatic element in the centric region of the chromosome.

ORIGIN AND STRUCTURE OF w^{vc}

The structure of the stable X^{c2} ring chromosome, as observed in the salivary glands by SCHULTZ and CATCHESIDE (1937) is consistent with the hypothesis that it arose by union of the distal end of one arm of an attached-X chromosome with the proximal region of the other to produce a minute deficiency for the tip of the X chromosome and a duplication for the proximal heterochromatin. After X-raying X^{c2} CATCHESIDE (CATCHESIDE and LEA 1945) recovered a chromosome which retained the ring structure but exhibited a variegated white Notch phenotype. Subsequent observations of the phenotype produced by this chromosome have shown that the variegation is enhanced at 18°C and suppressed by the addition of a Y chromosome, and that a spreading effect of variegation extends from the w^+ to the rst^+, spl^+, and N^+ loci. These results are typical of the V-type position effects produced by rearrangements transposing these genes from their normal position to a situation adjacent to the heterochromatin of the centromere region (LEWIS 1950). The fact that the phenotype produced by the y^+ locus shows no variegation indicates that a break occurred between the y^+ and w^+ loci (fig. 1). Cytological ex-

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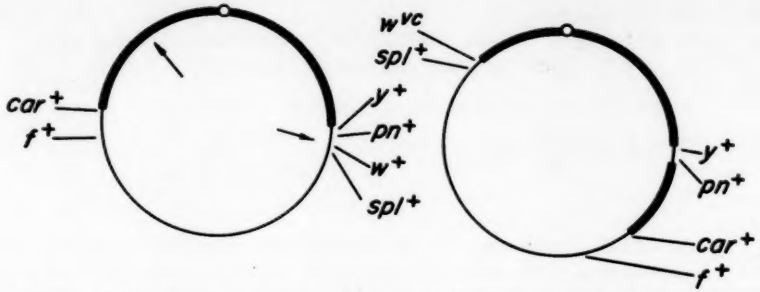

FIGURE 1.—The structure of X^{c2} and its inverted derivative, w^{vc}. The diagrams indicate euchromatin as a fine line, heterochromatin as a heavy line, the centromere as a small open circle, and the position of breaks by arrows within the X^{c2} chromosome. The position of the heterochromatic break and the relative lengths of heterochromatin and euchromatin are arbitrarily designated.

amination of this chromosome in the salivary gland nuclei reveals a long inversion configuration with the euchromatic breakpoint between the bands 3C1 and 3C5–6. Accordingly, this chromosome may be symbolized $In(1)X^{c2}$, w^{vc} and is conveniently abbreviated w^{vc}. It is not known whether the instability of w^{vc} arose as a concomitance of its inverted structure since the first record of its unstable nature appeared several years after its origin (GRIFFEN and LINDSLEY 1946).

MANIFESTATIONS OF w^{vc} INSTABILITY

In addition to the regular offspring expected from crosses involving ring chromosomes, gynandromorphs and X0 males occur very often in crosses of unstable w^{vc} (Crosses I–III, table 1). Gynandromorphs result from w^{vc} loss during the early indeterminate cleavages as shown by the extent and distribution of rod male tissue of mutant phenotype in otherwise wild type females. Gynandromorphs with noncontiguous male areas were relatively rare, and those resulting from loss of the rod-X chromosome were extremely rare.

In Cross I recognition of X0 males was facilitated by the use of the sc^8·Y chromosome marked by y^+ (MULLER 1948). Regular males received this tagged Y from their father, whereas X0 males either received and subsequently lost the w^{vc} chromosome or arose from nullo-X-nullo-Y sperm. Although nullo-X-nullo-Y sperm would be produced by primary nondisjunction in the male, this process makes no significant contribution to the X0 male class since primary exceptional rod/w^{vc}/Y daughters occur only rarely, if ever, among the progeny of w^{vc} males. In Crosses II and III where the w^{vc} chromosome is introduced by the female parent, X0 males were distinguished by their patroclinous phenotype. The origin of these patroclinous males from nullo-X eggs can be considered an alternative to their origin by complete loss of the maternal w^{vc} chromosome from w^{vc}/rod zygotes. There are two mechanisms which might give rise to nullo-X eggs from w^{vc}/rod females, namely, primary nondisjunction and double first anaphase bridges resulting from 4-strand double exchange tetrads (STURTEVANT and BEADLE 1936). These processes are probably of negligible consequence for the reasons that (1) w^{vc}/*dl-49* females produce less than 1% primary

TABLE 1

The results of typical crosses with the unstable w^{vc} *chromosome (I-III) compared with the stable* w^{vc} *(IV, V) and* X^{c2} *(VI, VII) chromosomes. Calculations of ring loss and dominant lethal frequencies are explained in the text*

Cross	Female parent	Male parent	Ring male	Rod male	Rod / Rod female	Ring / Rod female	Gynandromorph	X0 male	Frequency of: Ring loss	Frequency of: Dominant lethals	Total
Ia	*y w spl sn³*	w^{vc}, *f*/sc^8·Y	—	4808	—	2196	326	517	0.18	0.37	0.54
b			—	2469	—	743	151	179	0.13	0.57	0.70
IIa	$\frac{w^{vc}, f}{dl\text{-}49, y\ Hw\ m^2\ g^4}$	*y w spl sn*³/Y	97	601	637	241	62	91	0.24	0.38	0.62
b			162	613	858	280	38	84	0.14	0.53	0.67
c			97	1211	1295	140	106	181	0.22	0.67	0.89
IIIa	$\frac{w^{vc}, f}{y\ w\ spl\ sn^3}$	*dl-49, y Hw m² g⁴*/Y	365	710	1037	855	8	22	0.03	0.15	0.18
b			86	615	713	156	78	58	0.19	0.59	0.78
IV	$\frac{y\ w\ spl\ sn^3}{dl\text{-}49, y\ v\ f\ car}$	w^{vc}, *f*/sc^8·Y	—	2846	—	3022	0	1	—	—	—
V	$\frac{w^{vc}, f}{dl\text{-}49, y\ v\ f\ car}$	*dl-49, y w* lz^s/sc^8·Y	221	1446	1684	1649	0	2	0.00	0.02	0.02
VI	$\frac{y\ w\ spl\ sn^3}{dl\text{-}49, y\ v\ f\ car}$	X^{c2}, *cv v f*/sc^8·Y	—	9036	—	8441	83	72	0.02	0.05	0.07
VII	$\frac{X^{c2}, cv\ v\ f}{dl\text{-}49, y\ v\ f\ car}$	*dl-49, y w* lz^s/sc^8·Y	2703	1582	2864	2666	48	29	0.03	0.04	0.07

exceptional daughters and (2) no double crossovers are recovered from w^{vc}/*dl-49* heterozygotes. Elimination of primary nondisjunction and first anaphase bridge configurations as prime sources of nullo-w^{vc} gametes suggests that, if meiotic loss occurs, it is the result of the same process which operates during the cleavage mitoses. Therefore, regardless of their germinal or somatic origin, X0 males should be regarded with gynandromorphs as a class arising from w^{vc} loss.

One half of all gametes produced by w^{vc}/Y males should carry the w^{vc} chromosome and should be represented in the progeny of these males by the sum of females plus gynandromorphs and X0 males. However, comparison of this total with the number of regular males bearing the sc^8·Y shows a gross deficiency of the w^{vc} class instead of the 1:1 expectation (Cross I). In order to determine the source of this deficiency, counts were made of eggs from pair matings of *y v* females to w^{vc}/sc^8·Y males. The

TABLE 2

Comparison of dominant lethal frequency with the deficiency of presumptive w^{ve} *zygotes observed from pair matings of* y v *females to* w^{ve}/sc^{8}·*Y males. The results from each mating were obtained from five consecutive 24-hour subcultures*

Mating No.	Egg counts			Adult counts					
	No. laid	No. lethal	Dominant lethal frequency	Rod male	w^{re} female	Gynandromorph	X0 male	w^{ve} Zygote deficiency	w^{re} Lethal frequency
1	262	79	0.30	129	15	5	12	97	0.75
2	243	68	0.28	113	11	7	10	85	0.75
3	252	67	0.27	129	29	11	4	85	0.66
4	273	71	0.26	139	14	5	13	107	0.77
5	259	58	0.22	124	24	8	16	76	0.61
6	261	58	0.22	125	46	11	14	54	0.43
7	228	44	0.19	108	41	8	14	45	0.42
8	182	20	0.11	82	40	2	16	24	0.29
9	172	15	0.09	80	52	5	10	13	0.16
10	258	22	0.09	113	70	8	15	20	0.18
11	238	17	0.07	112	73	8	14	17	0.15
12	219	14	0.06	105	69	10	10	16	0.15
13	94	1	0.01	46	44	0	0	2	0.04

number of eggs which failed to hatch agreed closely with the w^{re} adult deficiency measured in the same cross (table 2). The results of this experiment show that the w^{ve} deficiency is primarily attributable to zygotic or embryonic lethality rather than gametic or postembryonic loss. When the female parent contributes the w^{re} chromosome it is also clear that the number of presumptive w^{re}/rod zygotes recovered as adults is strikingly reduced from the expected number based on rod/rod females. These observations lead to the conclusion that instability of the w^{re} chromosome is characterized not only by its loss but by dominant lethality as well.

An estimate of the frequency of zygotes affected by w^{re} instability can be made on the assumption that w^{re} gametes and gametes bearing the w^{re} homolog (either rod-X or Y) are produced in a 1:1 ratio. Thus from the cross of rod/rod females by w^{re} males, the rod male class provides an estimate of the number of w^{re} gametes produced; consequently, (Gy + X0 ♂) ÷ rod ♂ represents the proportion of w^{re}/rod zygotes in which loss occurs, and (rod ♂ − w^{re} ♀ − Gy − X0 ♂) ÷ rod ♂ gives the dominant lethal frequency among w^{re}/rod zygotes. In the reciprocal cross of w^{re}/rod females by rod males, the accuracy of these estimates is probably improved by substituting the rod/rod female class for the rod male class as the basis of expectation. No other attempt to correct for differential viability not associated with w^{re} instability has been made, and in general the effect of errors from this source is not sufficient to alter conclusions based on these estimates. It can be seen on inspection of the estimates of w^{re} loss and lethality listed for Crosses I–III (table 1) that there is considerable variability associated with both attributes of w^{re} instability, even in the same cross performed at different times (cf. Cross Ia with Ib, etc.). Although Crosses I and II are not strictly reciprocal, there appears to be no essential difference in the pattern of w^{re} behavior dependent upon maternal or paternal origin.

In controlled experiments with sib w^{rc}/rod females in comparison tests, it was demonstrated that the total instability is not affected by autosomal or sex-linked modifiers or by addition of a Y chromosome. However, w^{rc} instability is significantly decreased at 18°C as compared with 26°C developmental temperature, and the F_1 w^{rc} instability increases with increasing age of w^{rc} mothers. These results supported the conclusion that the variability is an inherent property of the w^{rc} chromosome which is subject to environmental or physiological modification (HINTON 1954).

Crosses IV and V (table 1) illustrate the behavior of a w^{rc} chromosome which became stable in stock culture; this chromosome is more stable than the standard stable X^{c2} chromosome (Crosses VI and VII) by the criteria of loss and lethality. Three other cases of w^{rc} stabilization have been observed under controlled conditions. Stabilization resembles a mutational event in the w^{rc} chromosome since only one individual of a generation exhibits the change from high instability to near or complete stability and no segregating modifiers are demonstrable. In all cases, the original w^{rc} phenotype and ring structure are maintained. No instances of the reverse change from the completely stable to the unstable condition have been recorded, although it has been possible to select highly unstable w^{rc} chromosomes from lines characterized by low instability.

THE OCCURRENCE AND BEHAVIOR OF w^{vc} DUPLICATIONS

From crosses of various yellow, attached-X females to unstable w^{rc} males, a few yellow mosaic daughters were obtained from which five different free duplications derived from the w^{rc} chromosome were isolated. Examination of larval brain smear metaphases showed that four of these duplications are small ring chromosomes (one duplication was lost before cytological examination could be made). The euchromatic composition of each of the duplications, determined in tests of their ability to cover various mutant loci, is limited to the loci immediately adjacent to the heterochromatin on one or both sides of the centromere (Part A of table 3 and fig. 1). The frequency of yellow mosaics produced from the cross of *Dp*/*y w f*/Y females by *dl-49*, *y w v f car*/Y males varies with the particular duplication and ranges from 100% down to 5.4% (table 4). This lower frequency is comparable to that given by *Dp(1;f)*X^{c2} which is a small ring-shaped duplication derived from X^{c2}. The size of mosaic areas also varies widely and is clearly correlated with the mosaic frequency; the modal size of yellow spots ranges from a few hairs for *Dp*(w^{rc})*6094a* and *Dp(1;f)*X^{c2} to over half the hypodermis for *Dp*(w^{rc})*6101*. If the duplication is one carrying the w^{rc} locus, mosaicism for eye pigmentation is registered in accordance with the yellow mosaicism of the hypodermis surrounding the eye.

SCHULTZ reported (BRIDGES and BREHME 1944) that the frequency of yellow mosaicism produced by *Dp(1;f)*X^{c2} could be partially suppressed by the addition of a Y chromosome, suggesting a position effect component of the mosaicism. This effect has been tested with *Dp*(w^{rc})*6094b* which, when present in eye tissue, permits detection of the Y chromosome through the latter's effect on the position effect variegation of the w^{rc} locus of the duplication. Among *64 Dp*(w^{rc})*6094b/dl-49*, *y w v f car*/0 males, 27 were mosaic, while 102 of 198 *Dp*(w^{rc})*6094b/dl-49*, *y w v f car*/Y males were mosaic. Since these frequencies are not significantly different, position

TABLE 3

The euchromatic constitution of duplications derived from the unstable w^{re} *chromosome. The duplications listed under Part A were recovered from attached-X females; those listed under Part B were observed in sterile gynandromorphs or X0 males*

Presumptive zygotic genotype	Identification or number of duplications	Fraction of tested duplications covering mutant loci										
		y	*w*	*spl*	*sn*^{3}	*lz*^{a}	*v*	*m*^{2}	*g*^{4}	*f*	*car*	*bb*
Part A												
y f:= /w^{re}, *B*	*Dp*(w^{re})*4097*	1/1	1/1*	1/1*						0/1		
y w/w^{re}, *f*	*Dp*(w^{re})*5279*	1/1	1/1	0/1	0/1		0/1				0/1	
y w *f*/w^{re}, *f*	*Dp*(w^{re})*6094a*	1/1	0/1	0/1	0/1							
	Dp(w^{re})*6094b*	1/1	1/1	0/1	0/1		0/1				0/1	0/1
	Dp(w^{re})*6101*	1/1	0/1									
Part B												
y w spl sn^3 *(bb)*/w^{re}, *f*	9	9/9	5/7	0/7	0/9							0/2
dl-49, y Hw m^2 *g*^{4}/w^{re}, *f*	7	7/7						0/4	0/3			
dl-49, y w lz^a/w^{re}, *f*	6	6/6	4/4			0/4						
dl-49, y w v f car/w^{re}, *(f)*	6	6/6	2/3				0/1			0/2	0/1	
dl-49, y v f car/w^{re}, *f*	2	2/2					0/1				0/1	
w^{m4}, *y w sn m*/w^{re}, *f*	1	1/1			0/1							
Total	36	36/36	14/19	1/11	0/13	0/4	0/4	0/4	0/3	0/3	0/4	0/3

* Constitution inferred from Confluens phenotype of *y f*:= females.

TABLE 4

The frequency of yellow mosaics produced from the cross of Dp/y w f/Y *females by* dl-49, y w v f car/Y males

Duplication	*Dp/y w v f car* ♂♂			*Dp/y w f* ♀♀		
	Nonmosaic	Mosaic	Percent mosaic	Nonmosaic	Mosaic	Percent mosaic
Dp(1;f)X^{c2}	18	1	5.3	213	21	9.0
Dp(w^{re})*4097*		Lethal		97*	97*	50.0
Dp(w^{re})*5279*		Lethal		0	57	100.0
Dp(w^{re})*6094a*	403	53	11.6	565	32	5.4
Dp(w^{re})*6094b*	133	129	49.2	270	192	41.6
Dp(w^{re})*6101*	0	35	100.0	0	85	100.0

* These females carried the *y f*:= double-X chromosome of MULLER.

effect variegation probably does not contribute to the mosaicism displayed by the w^{re} duplications.

Among the progeny of crosses involving unstable w^{re} and various *y* rod chromosomes, very rare gynandromorphs and X0 males have been observed in which part or all of the male tissue was phenotypically wild type rather than yellow as expected; in those instances where the rod also carried the *w* allele, a variegated male eye phenotype was sometimes found in conjunction with the surrounding wild type male hypodermis. Despite their occurrence in sterile individuals, sufficient information is available to interpret these phenotypes on the basis of small w^{re} duplications comparable to those described in the preceding paragraphs. The data itemized

below are pertinent to the origin, frequency, and constitution of these duplications. (1) Of 31 cases, 13 occurred among 13,929 gynandromorphs and 18 were found among 12,360 X0 males. The duplications which occurred in gynandromorphs must have been of somatic origin since the zygote received a whole w^{rc} chromosome; although this interpretation may also be applicable to those duplications observed in X0 males, it is possible that they were of gametic origin. (2) The frequency of w^{rc} duplication occurrence was higher when the w^{rc} chromosome was introduced by the male parent (13 cases out of 3,772 individuals) than when it was of maternal origin (18 cases out of 22,527) individuals. The reason for this difference is not apparent. (3) In 19 of the 31 cases, the duplication was present in only part of the X0 male tissue, the remainder being of the mutant phenotype expected from the particular rod used. Mosaicism of these duplications may be either a consequence of their formation or indicative of loss after formation (see discussion). (4) The genetic constitution of the duplications was determined with reference to the mutant alleles carried by the rod chromosome, provided that the duplication was present in the tissue affected by the particular mutant. Examination of table 3, which summarizes the constitution of these 31 duplications (Part B) and the 5 cases described earlier (Part A), shows that all 36 duplications included the y^+ locus, that 14 included and 5 excluded the w^{rc} locus, that only 1 of 11 probably carried the spl^+ locus, and that no other mutant locus over the entire euchromatic length of the X chromosome was covered by any of the duplications. These data, considered in reference to the structure of the w^{rc} chromosome, suggest that these 36 duplications comprise in common the w^{rc} centromere and part of the adjacent heterochromatin bounded on either side by the w^{rc} and y^+ markers; in addition, some fraction of the heterochromatic segment marked by y^+ and car^+ may be included in the duplications.

This analysis of w^{rc} duplications leads to two conclusions regarding w^{rc} behavior. First, the production of the duplications must be considered a fourth manifestation of w^{rc} instability. Second, the maintenance of instability by some of the recovered duplications shows that control of the instability mechanism must reside in that part of the w^{rc} chromosome present in the duplications.

DISCUSSION AND INCIDENTAL RESULTS

Two distinct mechanisms have been previously proposed to account for ring chromosome loss during the cleavage divisions of *Drosophila melanogaster*. The first of these, advanced by MORGAN (1926), is exclusion of the ring chromosome from telophase nuclei as a consequence of lagging on the anaphase spindle. The second means by which the ring chromosome might be lost from a cleavage nucleus is through the formation of an anaphase bridge. Thus GRIFFEN and LINDSLEY (1946) suggested that the w^{rc} chromosome might form anaphase configurations composed of interlocked single rings as the consequence of a 360° shift in the plane of chromosome reduplication. This result is similar to that produced by two progressive exchanges between sister chromatids of a ring chromosome at the time of its reduplication as envisaged by MCCLINTOCK (1938) in her cytogenetic study of somatic ring chromosomes in maize. A dicentric double ring anaphase bridge would result from a single exchange between sister chromatids.

Evidence in favor of the anaphase bridge hypothesis of ring chromosome loss has

been presented by BRAVER and BLOUNT (1949) who found bridge configurations in 12 and 22% of the larval brain anaphases of $X^{rc2}/dl\text{-}49$ and $w^{rc}/dl\text{-}49$ females, respectively. In conjunction with the present study, aceto-orcein smear preparations were made of the brains from unstable w^{rc} and stabilized w^{rc} male larvae. Bridges were observed in 10.1% (N = 179) of the late anaphases and early telophases of the unstable w^{rc} material whereas the stabilized w^{rc} produced 1.6% (N = 310) bridges. Although many rather diagrammatic examples of dicentric double ring bridges were observed in these preparations and in w^{rc} female brain smears, no unequivocal figures of interlocked single ring bridges were recorded. The disagreement between this observation and the report of BRAVER and BLOUNT that most of the bridges in their preparations were composed of interlocked single rings may not be surprising in view of the cytological difficulty of the material. Another interesting cytological feature is that X0 cells are only very rarely found in larval brains of unstable w^{rc}/rod females, which indicates that the result of anaphase bridge formation at this stage of development is not ring chromosome loss. This difference in the fate of anaphase bridges might be correlated with the syncytial structure of the early embryo as compared with the cellular structure of the larval brain. It is evident that the relation of anaphase bridge configurations in larval brain mitoses to those hypothesized to occur during the cleavage divisions remains to be clarified.

The analysis of w^{rc} behavior requires consideration of an hypothesis to account for the production of gynandromorphs, X0 males, dominant lethals and unstable duplications derived from the w^{rc} chromosome. In addition, such an hypothesis should account for the changes in the degree of w^{rc} instability and thus provide a clue to the difference in behavior of unstable w^{rc} and the typical stable ring-X chromosomes. Although gynandromorphs and X0 males might arise from anaphase lagging, this behavior is not sufficient to produce dominant lethals or w^{rc} duplications which appear to require breakage of the chromosome. On the other hand, anaphase bridge formation furnishes an adequate model of the w^{rc} instability mechanism.

The first possibility to be discussed is that the initial bridge configuration is a dicentric double ring of the type produced by a single exchange between sister chromatids. Such a bridge would appear to present ample opportunity for loss since at least two breaks are required to deliver any part of the bridge to a daughter nucleus. Given the required breaks in each member of the bridge, most of the products would be lethal because of genetic unbalance arising either at this time or following a bridge breakage fusion cycle. The only products which could be expected to survive would be those reconstituting the original euchromatic composition, those of the original composition plus or minus a small euchromatic segment, and small free duplications. Although changes in the heterochromatic constitution of the chromosome would probably not cause serious viability defects, these events would not ordinarily be recognized.

Alternatively, the primary anaphase bridge might be composed of interlocking single ring chromosomes. It seems unlikely that dominant lethals or w^{rc} duplications could arise directly from interlocked rings since a single break would suffice to resolve the interlock. If however, the break is followed by torsional restitution or sister chromatid union initiating a bridge breakage fusion cycle, the consequences of inter-

locked ring bridges become indistinguishable from those of a primary bridge formed by a dicentric double ring. Although it is generally assumed that chromatid type bridge breakage fusion cycles contribute to the production of radiation induced dominant lethals in Drosophila, there is no direct evidence on this point; in Habrobracon, however, WHITING (1945) has observed bridge configurations in cleavage mitoses following irradiation of oocytes. In any event, the anaphase bridge model of w^{rc} instability may not require the operation of bridge breakage fusion cycles unless the primary bridge is of the interlocked ring type.

No special crosses have been designed to detect w^{rc} chromosomes having hyperploid euchromatic segments, but deficient w^{rc} chromosomes would have been detectable in routine crosses involving rods bearing mutant markers. Possible examples of deficient w^{rc} chromosomes may be represented by nine $w^{rc}/y\ w\ \ldots$ females which displayed completely white rather than the expected white-variegated eyes; in eight of the nine cases the w^{rc} chromosome was introduced by the male parent. Since one of the exceptional females had a small sector of variegated tissue in one eye, and since four of the nine produced only typical variegated w^{rc} offspring, it is suggested that their phenotypes were the results of early somatic events. One of the "white" w^{rc} females was sterile. The remaining four transmitted w^{rc} chromosomes which continued to give a white eye phenotype in subsequent generations and which presented the normal ring configuration in larval brain metaphases. The deficiency hypothesis for the origin of these altered w^{rc} chromosomes is supported by the fact that two of them are male lethal. It appears unlikely that the two cases which are male viable might also represent deletions of the w^{rc} locus, and thus mutation of w^{rc} to w remains an alternative interpretation of these cases. No examples of w^{rc} chromosomes apparently deficient for other loci have been recorded.

The anaphase bridge hypothesis of w^{rc} instability predicts the formation of small free w^{rc} duplications as bridge breakage products. Some of or all those duplications observed in X0 males may have been derived from bridges formed at the second meiotic division in either sex. The interpretation of the recovery of those duplications which were observed in gynandromorphs is less direct. Since the reciprocal product of the cleavage bridge, if included in the sister telophase nucleus, would be lethal by all expectations, it becomes necessary to assume that either the reciprocal product was lost at the next mitosis, or that it behaved as a nuclear rather than zygotic lethal. A fourth possibility, suggested by the recovery of both *Dp*(w^{rc})*6094a* and *Dp*(w^{rc})*6094b* from a single attached-X female, is that two small duplications are produced simultaneously, one at each end of the bridge.

The simultaneous incorporation of both markers bounding the w^{rc} centromere region rules out the origin of w^{rc} duplications from double ring dicentrics formed by centromere misdivision since this event would place both y^+ loci at one pole of the bridge and both w^{rc} loci at the opposite end. The euchromatic constitution of the w^{rc} duplications may also have some bearing on the distribution of break points in double ring dicentrics. Clearly, the amount of euchromatin recoverable in a duplication is limited by viability considerations. However, this should not influence the observation that 14 of 19 tested duplications included the w^{rc} locus whereas only 1 of 11 apparently included the *spl*$^+$ allele separated from w^{rc} by only 4 or 5 salivary chro-

mosome bands. Secondly, although duplications carrying the w^{rc} locus but lacking the y^+ locus could have been detected, all 36 duplications included y^+. It may be worth repeating in this context that only w^{rc} chromosomes apparently deficient for the w^{rc} locus were observed although others could have been recognized. These results suggest the possibility that double ring dicentrics tend to break at localized positions.

Anaphase bridge formation also provides an adequate basis for understanding the instability exhibited by some of the duplications. Since there has been no suggestion of lethality caused by unstable w^{rc} duplications beyond that expected from duplicated euchromatin *per se*, it is possible that bridges formed by small rings are more susceptible to loss than to breakage as McCLINTOCK (1938) found to be the case with the small rings in maize. One of the strongest bases for McCLINTOCK's suggestion that sister-strand exchange is the primary cause of bridge formation by maize rings was her observation that bridge frequency is dependent on ring size. Such a direct comparison has not been possible between w^{rc} and its small ring counterparts.

On the basis of the instability and genetic constitution of the w^{rc} duplications, it was concluded that control of w^{rc} instability must reside in that part of the chromosome which is chiefly heterochromatic. Inclusion of the euchromatin marked by the y^+ allele may be a necessary, but not sufficient, factor in controlling w^{rc} instability; inclusion of the w^{rc} locus is clearly not necessary. If it is considered that instability of the w^{rc} chromosome is dependent on the dosage of some heterochromatic element of the w^{rc} centromere region, then the changes in degree of w^{rc} instability may be explained in terms of anaphase bridge formation. Either increases or decreases in the dosage, or complete loss, of this element might be represented in single rings derived from double ring dicentrics, and these changes would be reflected by altered instability of the recovered rings. It is neither required nor excluded that the events leading to the formation of anaphase bridges be restricted to that segment of the w^{rc} chromosome which controls its instability.

Although the results of this analysis are satisfied by the hypothesis of anaphase bridge formation, they provide little information relevant to the time, frequency, or cause of this event. Only gynandromorphs and certain w^{rc} duplications demand their origin from cleavage events; X0 males, dominant lethals and other w^{rc} duplications might be the consequence of dicentric double ring formation at anaphase II of meiosis as well as during the subsequent cleavage mitoses. Bridge formation at different times in embryonic development is clearly illustrated by the wide range of mosaic spot sizes produced by loss of w^{rc} duplications. Since anaphase bridges might be formed with different probabilities in any of several successive mitoses, and because of potential variables determining whether a bridge will break or be lost, it seems doubtful that the observed w^{rc} loss and lethal frequencies can be quantitatively related to frequencies of cytological events. In general, w^{rc} behavior appears to parallel that of maize rings; however, the evidence presented here does not demonstrate the occurrence of sister-strand exchange in the unstable w^{rc} chromosome.

SUMMARY

The structure of *In(1)*X^{c2}, w^{rc}, an unstable ring-X derived from the stable X^{c2} chromosome of *Drosophila melanogaster*, is described. Instability of the w^{rc} chro-

mosome is manifested by the frequent production of gynandromorphs, X0 males, and dominant lethals among w^{vc}/rod zygotes. Observations on unstable small ring duplications derived from the w^{vc} chromosome suggest that some heterochromatic element of the w^{vc} centromere region is responsible for w^{vc} instability.

The consequences of w^{vc} instability can be interpreted on the hypothesis that the unstable ring chromosome forms dicentric double ring configurations at anaphase of the cleavage mitoses and perhaps of the preceding meiotic division as well. Gynandromorphs and X0 males register loss of the dicentric, whereas dominant lethals arise by inclusion of bridge breakage products in the cleavage nuclei. The origin and continued instability of w^{vc} duplications are also explicable in terms of anaphase bridge formation, and it is postulated that changes in the degree of w^{vc} instability may be related to heterochromatic duplication or deficiency products of bridge breakage.

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Page 139, line 1, for "1:4:4" read "4:4:1".
Page 142, diagram, for "$S_5 \rightarrow S_6$" read "$S_5 \leftarrow S_6$".
Page 151, diagram, for "$S_6 \rightarrow S_8$" read "$S_6 \leftarrow S_8$".

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BEARING ON HEREDITY AND VARIATION

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